UNIVERSITY OF ALBERTA

PLASMA LEPTIN AT REST, DURING FASTING AND AFTER EXERCISE TRAINING IN PEOPLE WITH SPINAL CORD INJURY

By

Justin Y. Jeon

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Faculty of Physical Education and Recreation

Edmonton, Alberta

Fall, 2003

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisisitons et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-612-87998-4
Our file Notre référence
ISBN: 0-612-87998-4

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou aturement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this dissertation.

While these forms may be included in the document page count, their removal does not represent any loss of content from the dissertation.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de ce manuscrit.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

University of Alberta

Library Release Form

Name of Author: Justin Y. Jeon

Title of Thesis: Plasma leptin at rest, during fasting and after exercise training in people with spinal cord injury

Degree: Doctor of Philosophy

Year this Degree Granted: 2003

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purpose only.

The author reserves all other publications and other rights in association with the copyright of the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Justin Y. Jeon
The Steadward Centre
W1-67 Van Vliet Com
University of Alberta
Edmonton AB
T6G 2H9

August 19, 2003

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "PLASMA LEPTIN AT REST, DURING FASTING AND DURING EXERCISE TRAINING IN PEOPLE WITH SPINAL CORD INJURY" submitted by JUSTIN Y. JEON in partial fulfillment of the requirements for the degree of Doctorate of Philosophy.

Dr. Vicki Harber
Supervisor

Dr. Robert Steadward
Co-Supervisor

Dr. Gordon Bell

Dr. Linda McCargar

Dr. Garry Wheeler

Dr. Dan Syrotuik

Dr. Jeffery McCubbin

Thesis approved by the examining committee on August 20, 2003

ABSTRACT

The purpose of this dissertation was to examine the following in spinal cord injured (SCI) individuals: 1) the relationship between circulating leptin levels, body composition, and resting metabolic rate (RMR) (Study one), 2) the leptin response to a 36-hr fast (Study two) and 3) the effect of a 12-week FES-Rowing training program on plasma leptin levels (Study three)

Study one showed that the SCI group had 105% higher resting plasma leptin levels compared to the able-bodied (AB) group ($P=0.004$) when BMI was controlled for. Plasma leptin levels correlated with RMR in the AB group ($r=.90$, $P=0.006$), but not in the SCI group ($r=.44$, $P=0.43$), which suggests the loss of sympathetic nervous system-mediated RMR regulation in people with SCI. Study two showed that plasma leptin levels significantly decreased during 36 hr of fasting by 38.6% and 48.8% in SCI and AB, respectively, with the leptin reduction being delayed in the SCI group. This result suggests that people with SCI still responded to an energy imbalance with a decline in leptin, however, this delay in reduction may be due to an impaired SNS.

Following 12 weeks of FES-Rowing training, fat mass (4.58%, $P=0.14$) and plasma leptin levels (33%, $P=0.078$) declined (Study three) but these changes were not significant. Despite the small sample size in this study ($N=5$), plasma glucose levels decreased after the training ($P<0.05$). This reduction is clinically important since plasma glucose levels are well known markers of glucose metabolism and type 2 DM.

Overall, people with SCI have elevated plasma leptin levels compared to the AB population. Due to an impaired SNS, the relationship between leptin levels and RMR is not related in the SCI compared to the AB group. As a result of these changes, the risk of developing obesity may be increased. Study three demonstrated the possible benefits of exercise training as plasma leptin, glucose and fat mass were reduced after 12 weeks

of FES-Rowing training. Therefore, regular exercise training is recommended for people with SCI to alleviate increased fat mass, obesity and obesity-related disorders.

Acknowledgement

"The fear of the Lord is the beginning of knowledge, but fools despise wisdom and discipline"

Proverbs 1:7

"For the message of the cross is foolishness to those who are perishing, but to us who are being saved it is the power of God. For the foolishness of God is wiser than man's wisdom, and the weakness of God is stronger then man's strength."

1 Corinthians 1:18, 25

First of all, I thank God for his mercy and grace. You are amazing God who has never stopped loving and teaching me. Thank you!

Also, this final product would not be possible without the assistance and support of many individuals.

Jina Kim, my beautiful wife. Thank you for all your support and sacrifice. I am so blessed and honored to be your husband. You are the best!

David Jeon, my beloved son. I am sorry for not playing with you enough. You are always a good kid and I am so proud of you not because you are smart or well-behaved but just because of who you are. Also, thank you for waiting for me when I came home late. You always threw your arms around me and welcomed me when I came home late.

My mom and dad. Words cannot describe how much you mean to me. Your never ending love and understanding towards me is overwhelming. Thank you!

Dr. Harber. Thank you for your endless support over the past five years. You were always there for me when I really needed you. I will never forget your encouragement and support. Thank you for your patience and kindness!

Dr. Steadward. You are an amazing man for whom I have the most respect. You always believed in me when I was not really deserving. You can never imagine how much you have taught me. Thank you very much!

Dr. Bell. You have been my committee member since I started my graduate program. You have molded me into what I am now as a researcher. Thank you very much for your constructive criticism and encouragement!

Dr. Wheeler. How can I thank you enough! You always believed in me even when I doubt my own ability. You are the man of encouragement. Thank you very much! I am looking forward to continuing our friendship in the years to come.

Dr. McCargar. Thank you very much for your insight! You were always helpful and never fell short of insightful questions and comments. Thank you very much!

Thanks should also go to the rest of my examining committee who were supportive and helpful along the way: Dr. Dan Syrotuik, Dr. Jeff McCubbin and Dr. Brian Maraj. Dr. Dan Syrotuik always saved me with his humor when I was stressed out with difficult questions during my candidacy and also my final defense. Dr. Brian Maraj is one of the kindest and genuine persons I have ever met. Thank you for your big hug! Dr. Jeff McCubbin. Thank you for being available as an external committee member.

I would like to thank the staff of The Steadward Centre, the best fitness and research centre for people with disabilities. Barbara Campbell, Ewen Nelson, Will Gill, Sandy Jacobson and Kim Lagace, Neil Taylor, Dries Hittinga and Jean Weeks always encouraged me and supported me. Especially, Barbara Campbell who was like a mom to me at The Steadward Centre from day one of my graduate study. Thank you mom!

Finally, I would like to thank all the participants who were willing to fast for 36 hours, exercise for 12 weeks and be poked many times. Without your willingness and sacrifice, it would have been impossible for me to complete these studies. Thank you all very much!

TABLE OF CONTENTS

CHAPTER ONE: (Introduction) 1

Purposes of Research 5

CHAPTER TWO (Review of Literature) 7

Review paper 1. 8
Review paper 2. 40

CHAPTER THREE (Study one) 55

Introduction 56
Methods 57
Results 57
Discussion 58

CHAPTER FOUR (Study two) 62

Introduction 63
Methods 64
Results 64
Discussion 65

CHAPTER FIVE (Study three) 70

Introduction 71
Methods 75
Results 79
Discussion 81

CHAPTER SIX (Summary, discussion and future direction) 107

APPENDIX A: Subject data (Data excluded from group analysis) 114

APPENDIX B: Participant information letter of study one 115

APPENDIX C: Participant information letter of study two 119

APPENDIX D: Participant information letter of study three 125

LIST OF FIGURES

CHAPTER ONE

Figure 1. Relationship between plasma leptin and fat mass in the SCI and AB groups. 58

Figure 2. Relationship between plasma leptin and RMR in the SCI and AB groups. 59

CHAPTER TWO

Figure 1. Plasma leptin response to 36 hr of fasting 65

Figure 2. Relationship between plasma leptin and RMR in AB and SCI groups 65

CHAPTER THREE

Figure 1. Effects of 12 weeks of FES-Rowing on fat mass in people with high lesion SCI 103

Figure 2. Effects of 12 weeks of FES-Rowing on plasma leptin in people with high lesion SCI 104

Figure 3. Effects of 12 weeks of FES-Rowing on plasma glucose in people with high lesion SCI 105

Figure 4. Effects of 12 weeks of FES-Rowing on plasma insulin in people with high lesion SCI 106

Figure 5. Correlation between fat mass and plasma leptin in people with high lesion SCI pre- and post-training 107

LIST OF TABLES

CHAPTER THREE (Study one)

Table 1.	Subject characteristics	57
Table 2.	Association between 12-h leptin and body Composition and hormones	58
Table 3.	Metabolic parameters	58
Table 4.	RMR	59

CHAPTER FOUR (Study two)

Table 1.	Characteristics of subjects	64
Table 2.	RMR	66

CHAPTER FIVE (Study three)

Table 1.	Subject characteristics	99
Table 2.	Physical, metabolic and hormonal parameters pre- and post-training in individual subjects	100
Table 3.	Exercise training data: total training energy expenditure, number of training sessions, changes in power output and VO2 peak	101
Table 4.	Physical, metabolic and hormonal parameters pre- and post-training in fat mass and fat mass maintained group	102

LIST OF ABBREVIATIONS

SCI	Spinal cord injury
AB	Able-bodied
FES	Functional electrical stimulation
DM	Diabetes mellitus
SNS	Sympathetic nervous system
DEXA	Dual –energy X-ray absorptiometry
FM	Fat mass
FFM	Fat free mass
BMI	Body mass index
RMR	Resting metabolic rate
VMH	Ventromedial hypothalamus
CV	Coefficient variation
WC	Waist circumference
AMPT	α-methyl-p-tyrosine methyl ester
GLUT	Glucose transporter
GH	Growth hormone
TG	Triglyceride
FFA	Free fatty acid
RIA	Radioimmunoassay
NPY	Neuropeptide Y
MCH	Melanin-concentrating hormone
JAK	Janus family of protein kinase
AMPK	Adenosine-5′-monophosphate-activated protein kinase
MAPK	Mitogen-activated protein kinase
STAT	Signal transducers and activator of transcription
AMPO	Arm-only power output max
ACC	Acetyl-CoA carboxylase
IRS	Insulin receptor substrate
PI3-K	Phosphatidylinositol-3-OH kinase
VO2	Oxygen consumption

CHAPTER ONE

INTRODUCTION

INTRODUCTION

People with a spinal cord injury (SCI) have a three to five times higher risk of developing type 2 Diabetes Mellitus (DM) (1). Possible reasons for the high risk of developing type 2 DM include changes in body composition (6, 14) and changes in paralyzed muscle characteristics after the SCI (3, 5). The changes in body composition include increased fat mass and decreased muscle mass (13). Increased fat mass is one of the risk factors for the development of type 2 DM (2).

However, it remains unclear why people with SCI are more prone to becoming obese. In order to prevent obesity-related disorders, such as type 2 DM, it is important to understand the mechanisms underlying increased obesity in people with SCI. The recently discovered hormone leptin has contributed to the acceleration of obesity research in the last 7 years (14). Administration of leptin decreases food intake and increases energy expenditure, resulting in body weight loss (7, 12). The central nervous system (CNS) is the major site of leptin action (8, 12). This action of leptin is partially mediated by the sympathetic nervous system (SNS) (4, 9). In people with SCI, the SNS is impaired and interrupted due to the spinal cord lesion (6, 12). Also, people with cervical and high thoracic SCI have a reduced sympathetic nerve activity, which is associated with development of obesity (10). These factors may explain the higher risk of obesity and type 2 DM in SCI. However, to our knowledge, basal leptin levels, effects of short-term fasting and /or exercise (acute exercise and exercise training) on circulating leptin levels in the cervical and the high thoracic (above T6) (high lesion) SCI have not been investigated. These findings will improve our knowledge of obesity and obesity-related disorders in people with SCI.

In this thesis, the following will be investigated in subjects with high lesion SCI: *1)* basal leptin levels and its association with the body composition and resting metabolic rate, *2)* the leptin response to short term fasting, and *3)* the leptin response to exercise training.

REFERENCE

1. Bauman WA, Spungen AM.. Disorder of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: A model of premature aging. Metabolism. 43:749-756, 1994.
2. Bjorntorp, P. Portal adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 10: 439-496, 1990.
3. Burnham R, Martin T, Stein R, Bell. G, Maclean I, Steadward RD. Skeletal muscle fibre type transformation following spinal cord injury. Spinal Cord 35: 86 -91, 1997.
4. Haque MS, Minokoshi Y, Hamai M, Iwai H, Horiuchi M, Shimazu T. Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. Diabetes. 48:176-1712, 1999.
5. Jeon JY, Weiss CB, Steadward RD, Ryan E, Burnham RS, Bell G. et al. Improved glucose tolerance and insulin sensitivity after electrical stimulation-assisted cycling in people with spinal cord injury. Spinal Cord 40:110-117, 2002.
6. Karlsson AK, Elam M, Friberg P, Sorensen FB, Sullivan L, Lonnroth P. Regulation of lipolysis by the sympathetic nervous system: A microdialysis study in normal and spinal cord injured subjects. Metabolism. 46:388-394, 1997.
7. Martin L, Jones P, Considine R. et al. Serum leptin levels and energy expenditure in normal weight women. Can J Physiol Pharmocol. 76: 237-241, 1998.
8. Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes. 48:287-291, 1999.
9. Satoh N, Ebihara K, Ogawa Y, Masuzaki H, Katsuura G. Sympathetic activation of leptin via the ventromedial hypothalamus-leptin induced increase in catecholamine secretion. Diabetes. 48:1787-1793, 1999.
10. Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E et al. Body fat and sympathetic nerve activity in healthy subjects. Circulation. 89:2634-2640, 1993.
11. Schmid A, Huonker M, Barturen JM, Stahl F, Schmidt-Trucksass A. Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. J Appl Physiol. 85:635-641, 1998.

12. Tang-Christiansen M, Havel PJ, Jacobs R et al. Central administration of leptin inhibit food intake and activates the sympathetic nervous system in Rhesus Macaques. J Clin Endocrinol Metab. 84: 711-717, 1999.
13. Wilmet E, Ismail AA, Heilporn A, Welraeds D, Bergmann P. Longitudinal study of the bone mineral content and of soft tissue composition after spinal cord section. Paraplegia. 33:674-677, 1995.
14. Zhang Y, Proenca R, Maffei M et al. Positioning cloning of the mouse obese gene and its human homologue. Nature. 372:425-432, 1994.

PURPOSES OF THE RESEARCH

Study 1:

Research objective

To identify basal leptin levels and determine the relationship between leptin levels, body composition and RMR in the SCI group.

Research Hypotheses

- Plasma leptin levels will be higher in the SCI group compared to AB group.
- Plasma leptin levels will positively correlate with RMR in the AB group but not in the SCI group.

Study 2:

Research objective

To determine the leptin response to 0h, 12h, 24h, and 36h of fasting in the SCI and AB groups.

Research Hypothesis

- Plasma leptin will decrease with a 36- hour fast in the AB group but not in the SCI group.

Study 3:

Research Objectives

1) To determine the effects of 12 weeks of FES-Rowing training on plasma leptin level in people with spinal cord injury.

2) To investigate whether exercise training induced plasma leptin change in subjects with spinal cord injury is independent of fat mass change.

Research Hypothesis:

- 12 weeks of FES-Rowing training decrease plasma leptin level in people with SCI.

CHAPTER TWO

REVIEW OF LITERATURE

REVIEW OF LITERATURE INCLUDES TWO REVIEW PAPERS

PAPER 1. Obesity, Type 2 DM and Exercise in people with SCI -Implication of sympathetic nervous system impairment in people with SCI

Submitted for publication at American Journal of Clinical Nutrition

PAPER 2. What are the true effects of exercise on leptin regulation and action? -Are we measuring what we intend to measure?

Submitted for publication at Journal of Clinical Endocrinology and Metabolism

REVIEW PAPER ONE

Obesity, Type 2 DM and Exercise in people with SCI:
Implication of sympathetic nervous system impairment in people with SCI

Introduction

People with SCI have an increased risk of developing type 2 DM (1-3). Bauman et al. (1) demonstrated that among 100 subjects with paraplegia or quadriplegia, 22% have type 2 DM as compared to only 6% in the able-bodied (AB) control group. Sixty-two percent of those with quadriplegia and 50% of those with paraplegia have abnormal glucose metabolism, compared to only 18% in AB controls. Increased obesity following SCI is one of the major contributors for the higher incidence of type 2 DM (4-10). People with SCI have a significantly higher percent body fat compared to AB controls even after the subjects are matched by age, weight and body mass index (BMI) (4-7).

The association of obesity with type 2 DM has been recognized for decades (8-10). Insulin resistance is a fundamental aspect of the etiology of type 2 DM (9). The risk of type 2 DM increases 11-fold as BMI increases from 20 to 30 kg/m^2 (10). Obesity represents an expansion of the adipose tissue mass, which then influences its own secretory products. Tumor necrosis factor-alpha (TNF-α) is over-expressed in adipose tissue of obese and insulin resistant animals and humans (11-12). TNF-α is known to impair insulin signaling, inhibit lipoprotein lipase (LPL), and stimulate lipolysis in adipocytes, leading to an increase in the circulation of nonesterfied fatty acids (NEFA) (11-13). The chronic elevation of free fatty acids (FFA) in the obese may contribute to the decreased uptake of glucose into the peripheral tissues (14-15), and reduce the insulin receptor substrate (IRS)-1-associated phosphatidylinositol (PI) 3-kinase activity (14). A defect in the PI-3-kinase mediated insulin signaling pathway causes insulin resistance in skeletal muscle, adipocyte, and liver (16). Obese individuals also show reduced GLUT-4 expression in the adipocytes and impaired insulin-stimulated translocation of GLUT-4 in the skeletal muscle, as a result of defective PI-3-Kinase-mediated insulin signaling (16-17).

Lesion level and percent body fat are strongly correlated in people with SCI (18-20). People with high and complete lesions have significantly higher occurrences of impaired glucose tolerance and type 2 DM than those who have low and incomplete lesions (2). As well, people with high and complete lesions lose more of their supraspinal regulatory controls and sympathetic nerve activity, resulting in a lower resting metabolic rate (RMR) (21-22). Lower sympathetic nerve activity and lower RMR

are also associated with obesity (23-26). When decreased energy expenditure is not compensated by decreased food intake, adiposity will increase in the SCI population.

It is of special interest, therefore, to understand the physiology of leptin in people with SCI whose SNS is impaired (18-20, 27, 28). The SNS plays a key role in the normal function of leptin and is required for the increase in glucose uptake (29-30), decrease in insulin secretion (31), and increase in energy expenditure (32-34). Despite its contribution to these key processes, basal leptin levels and the leptin production in response to fasting or exercise in people with high lesion SCI have not been investigated. In this review, we examine the following: 1) the role of leptin in body weight regulation, 2) exercise and leptin, 3) the role of SNS in leptin metabolism, 4) impairment of SNS in SCI, 5) leptin metabolism in people with SCI, and, 6) effects of exercise training on type 2 DM.

ROLE OF LEPTIN IN BODY WEIGHT REGULATION

Despite the medical problem of obesity throughout the world, one of the most interesting facts is the stability of body weight (35-36). Body weight is tightly regulated by a feedback loop, which maintains body weight homeostasis (36). Identification of the *ob* gene using positional cloning and the characterization of the hormone leptin has greatly increased our understanding of the pathophysiology of obesity and its related disorders (37-38). Leptin is produced by adipose tissue and affects food intake, energy expenditure, and neuroendocrine status (39). Leptin deficiency, as seen in *ob/ob* mice and human with congenital leptin deficiency, causes obesity (41-42). Lack of leptin in circulation is perceived in the hypothalamus as energy deficient status and the brain sends a signal to increase energy intake and to decrease energy expenditure, resulting in obesity (41-42). Conversely, the administration of leptin to *ob/ob* mice and children with congenital leptin deficiency decreases feeding, normalizes body fat, body temperature, and also corrects neuroendocrine abnormalities (37, 41, 42, 43). Studies report that food intake is reduced by 30-31% and glucose uptake increased up to 27% in response to leptin administration in leptin-treated animals compared to a control group (43). Although *ob/ob* mice have no leptin, it soon became apparent that leptin levels are almost universally raised in obese rodent and human, in contrast to what was initially predicted

(44-45). The finding that leptin circulates in plasma in proportion to the amount of fat mass, suggests that human obesity is related to leptin resistance rather than leptin deficiency.

There is also increasing evidence that leptin may regulate the neuroendocrine response to starvation (46-50). In lean individuals, plasma leptin concentrations decline markedly within the first 12 h of fasting, which may be important in regulating substrate metabolism and energy expenditure during early starvation (4, 46-50). During a four-week period of energy restriction, Weiss et al. (48) reported a 66% decline in the plasma leptin level after one week followed by a more gradual decline thereafter. However, a decrease in fasting-induced plasma leptin was prevented by maintaining euglycemia via glucose infusion during a 72-h fasting period (50). Leptin reduction during fasting will decrease energy expenditure and increase food intake and therefore, defends the body from excess energy expenditure in the face of limited energy intake (39-40).

EFFECTS OF AEROBIC EXERCISE ON LEPTIN

Leptin response to acute exercise

Following an acute bout of exercise, plasma leptin levels have been reported to decrease (51-53), increase (54), or remain unchanged (55-60). Activities such as 2 h of cycling exercise (53) a 20-mile treadmill run (57), and 60 min of cycling at 50% VO2 max (58) did not change leptin levels during or immediately following these protocols. On the other hand, other studies noted that a marathon run (61) and a 2 h run at 75% VO2 max (62) significantly decreased plasma leptin levels. The studies that observe the decreased leptin levels following physical activity often involve exercise of longer durations at higher intensities, resulting in higher energy expenditure (52, 53). Evidence of this comes from Landt et al. (53) who detected a 32% reduction in plasma leptin after a 101mile ultramarathon. In addition, Karamouzis et al. reported a 50.5% reduction in leptin levels after marathon swimming (25 km sea swimming, 6.5-10.5 hours). These data suggest that higher exercise energy expenditures resulting in higher negative energy balance are more likely to alter leptin levels.

Exercise stress vs Energy availability

In order to examine whether energy availability (energy intake minus energy expenditure) or exercise stress (everything associated with exercise excluding energy cost) influences plasma leptin levels, Hilton et al. (56) controlled energy intake and exercise energy expenditure in healthy young women and measured the diurnal leptin rhythm. These researchers found that low energy availability suppresses the 24 h mean and amplitude of serum leptin, whereas exercise stress by itself does not. However, Van Aggel-Leijssen et al. (63) reported that exercise with energy balance still decreases 24 h mean serum leptin compared to a non-exercise energy balance control. Van Aggel-Leijssen et al. (63) also reported that a disturbance in energy balance, caused by overfeeding on a day of relatively high physical activity increases the absolute amplitude of the 24h plasma leptin curve significantly. These conflicting results make it difficult to draw any conclusive decision whether an acute bout of exercise independent from negative energy balance would influence plasma leptin levels.

Factors influencing leptin production during exercise

Although a greater exercise-induced negative energy balance would most likely cause a reduction in plasma leptin, a number of discrepancies in the findings still exist. These discrepancies may be explained by hormonal response to intense and prolonged exercise. An intense bout of exercise increases both plasma cortisol and catecholamine levels (64-65). Cortisol is known to increase leptin production, while catecholamines are known to reduce plasma leptin production (66-68). During and immediately following an interval exercise at 76% of VO2max for 41 minutes (475kcal), Fisher et al. (54) reported that both cortisol and catecholamine levels were increased, but leptin levels were no different from baseline. Increased leptin levels were detected 139 min into the recovery period while catecholamine levels returned to baseline and cortisol levels remained significantly elevated (54). This finding may suggest influence of exercise induced catecholamine and cortisol changes on plasma leptin during and immediately after exercise. Also, intense exercise decreases insulin secretion through sympathetic innervation (69). A recent investigation demonstrated that the treatment of rat adipocytes with insulin increased tissue leptin content and secretion during a 2 h

incubation period (71). In addition, leptin release increased within 10 min of insulin administration, suggesting that insulin may act as a leptin secretagogue (72). Therefore, the conflicting results about leptin's response to exercise may be due to counter-regulatory responses during and immediately after exercise.

Knowing that elevation of catecholamine, insulin and cortisol decreased to the basal levels within a few hours from cessation of an exercise bout, it is interesting that studies that reported a decrease in plasma leptin levels following exercise have obtained samples 2h-48h post-exercise (51, 62, 74). Following exercise bouts of similar duration and intensity, Duclos et al. (51) detected a 30% reduction in plasma leptin 2 h after the cessation of the exercise but Hickey et al. (59) found no difference when samples were taken immediately following exercise. Additionally, Tuominen et al (62) reported a 34% reduction in plasma leptin levels 44 h after 2h running at 75% VO2max and Essig et al. (74) reported a 30% reduction in plasma leptin levels after 48 h after two treadmill exercise sessions with different energy expenditures (800 or 1,500 kcal) at 70% VO2max. Based on these findings, we may speculate that exercise with substantial energy expenditure causes leptin reduction, however, when leptin levels are measured during or immediately following exercise, exercise induced leptin reduction can be shadowed by counterregulatory hormones which influence leptin production. Thus, one of the possible reasons for the delayed decrease in plasma leptin levels may be related to the influence of exercise-induced changes on hormonal responses.

Leptin production vs Leptin clearance

Animal studies generally produce consistent results on the effects of exercise on leptin gene expression (75-77). Zheng et al. (77) reported that a single bout of exercise significantly decreased *ob* mRNA levels approximately 30% immediately and 3 h after exercise in male Sprague-Dawley rats. They also reported that 4 weeks of exercise training decreased the ob gene expression by 48% at 2 h after the last bout of exercise training compared to the control group. Bramlett et al. (75) reported that treadmill exercise until exhaustion (average 85.5±1.5 min) decreased leptin mRNA significantly in retroperitoneal fat. Moreover, the decrease in leptin mRNA after exercise resulted from stimulation of cyclic AMP-dependent protein kinase and beta-3 adrenergic receptors.

Norepinephrine as well as specific beta 3 adrenergic agonists reduce leptin expression in white adipose tissue. Intense exercise increases norepinephrine levels up to 14 fold, which in turn increases cyclic AMP-dependent protein kinase necessary to decrease exercise-induced leptin expression (78-79). Therefore, it can be speculated that the exercise-induced catecholamine increase may reduce leptin gene expression in these animals.

However, animal studies still produce conflicting results on the effects of exercise on plasma leptin levels (75, 80-81). Pagano et al. (80) reported a 30% reduction in plasma leptin after 30 min swimming exercise. On the other hand, Bramlett et al. (75) reported a 59% increase in circulating leptin level after 55 min of treadmill running. This conflicting result could be related to leptin clearance during exercise. While leptin gene expression solely represents leptin production, circulating leptin levels can be contributed to leptin clearance as well as leptin production. Fogteloo et al. (82) recently reported that 80% of the total body leptin clearance could be attributed to the kidney. Interestingly, intense exercise decreases blood flow to the kidney and thus may decease leptin clearance during intense exercise (83). Also, studies that have reported no change in leptin levels immediately after exercise report a reduction in plasma leptin levels 2 to 44 hours after the cessation of an exercise bout (51, 62, 74). This result may be related to a reduction in renal leptin clearance during exercise. In combination of the result from human and animal studies, it is evident that exercise can decrease leptin mRNA and leptin production in adipose tissue. However, due to the lack of control in leptin clearance and counter-regulatory hormones that effect leptin production during exercise, we may observe conflicting results on the effects of exercise on circulating leptin level.

Leptin response to chronic aerobic exercise

There is also considerable variation in the reported effects of chronic exercise on circulating leptin levels (84-92). Studies have demonstrated a reduction (84-88), or no change in plasma leptin following an exercise training program (90-92). The differences in these findings could be explained by concurrent weight loss with exercise training. Studies observed weight loss with training have also observed a reduction in plasma

leptin levels (85-87). Halle et al. (85) studied plasma leptin levels in 20 men with type 2 DM before and after six weeks of training (2,200kcal/wk). They reported that their exercise program caused a 4.3% weight loss and a 30% reduction in plasma leptin levels. Kohrt et al. (86) also reported a 4.9% loss in body mass and a 27.9% reduction in plasma leptin levels after nine months of training. These results are not unexpected, as leptin is known to be produced and secreted by adipose tissue and it is well documented that plasma leptin correlates with body fat mass (44, 45).

Is weight loss necessary for plasma leptin changes?

Although there is convincing evidence that exercise training that leads to weight loss will decrease plasma leptin levels, it is unclear whether exercise training-induced leptin reduction is independent of changes in fat mass. Hickey et al. (91) reported a reduction in plasma leptin level in females after 12 weeks of training (4d/wk, 1200 kcal/wk) despite stable fat mass. Perusse et al. (58) also reported a reduction in plasma leptin in obese males after 10 months of exercise training independent of changes in body fat mass. Furthermore, Okazaki et al. (87) also demonstrated that plasma leptin levels decreased in 14 subjects whose fat mass either did not change or slightly increased after a 9 month exercise intervention. Moreover, Ishii et al. (89) report a reduction in plasma leptin with the combination of diet and exercise, but not with diet alone after 6 weeks of exercise training. Ishii et al. (89) also standardized leptin level per kg fat mass and report that plasma leptin per kg fat mass was lower in the combination of diet and exercise group as compared to diet only group. This was found to be conversely so in a well-controlled study examining the independent effects of weight loss and exercise on plasma leptin in 52 obese men (90). Subjects were randomly assigned to four 12-week protocols: 1) control, 2) diet-induced weight loss, 3) exercise-induced weight loss, and 4) exercise with weight maintenance. They found that leptin was reduced in both the exercise- and diet-induced weight loss groups but not in the exercise with weight maintenance group. The authors suggest that exercise-induced leptin reduction is dependent upon weight loss. Kreamer et al. (92) also did not observe significant changes in plasma leptin level after exercise training in 16 overweight women under

weight stable conditions. Therefore, it is not yet clear whether exercise training induced leptin reduction is independent of fat mass change or not.

ROLE OF SYMPATHETIC NERVOUS SYSTEM IN LEPTIN METABOLISM

Role of Sympathetic Nervous System in leptin regulation

While the SNS-mediated fatty acid mobilization from adipose tissue have long been understood (93-94), it has recently become apparent that the SNS is also a key regulator of leptin production in adipose tissue (95-97). Sympathetic stimulation of white adipose tissue by sympathomimetic amines and cold exposure or fasting decreases gene expression and production of leptin (46, 67, 98). However, sympathetic blockade often increases circulating leptin and leptin gene expression, which suggest a tonic inhibitory action on leptin synthesis (99-100). The suggestion of a tonic inhibition of the leptin system by the SNS is strengthened by the result of the administration of α-Methyla-p-tyrosine (AMPT) or its more soluble methyl ester (AMPT-ME) on leptin. AMPT depletes tissue norepinephrine by inhibiting tyrosine hydroxylase, the initial and rate-limiting step in neuronal catecholamines synthesis (100). Administration of intraperitoneal AMPT-ME, (250mg/kg) increased both plasma leptin (15-fold) and leptin mRNA levels in interscapular brown adipose tissue and epididymal fat (99). Additional evidence by Pinkney et al. (100) demonstrates that infusion of a β-adrenergic agonist (isoprenaline) rapidly reduces circulating levels of leptin. As well, Commins et al. (68) illustrates that leptin's effects on gene expression in brown and white adipose tissue is dependent upon norepinephrine synthesis and secretion. We have recently demonstrated that people with SCI have significantly higher levels of circulating leptin after correction for body fat, which provides evidence for the presence of a tonic inhibitory adrenergic influence on leptin secretion in humans (4, 21).

Role of SNS in mediating leptin's metabolic effects

The SNS also mediates many of leptin's metabolic effects (29-34). Administration of leptin increases glucose uptake in the heart, brown adipose tissue and skeletal muscle, independent of changes in plasma insulin (29-30). However, glucose uptake in peripheral tissues is abolished after leptin injection when surgical or chemical sympathectomy is performed (30). Also, leptin's effect on glucose uptake was blocked by preventing the release of norepinephrine from sympathetic nerves with guanethidine

treatment, indicating the sympathetic innervation is responsible for these effects (29). Leptin administration also suppresses glucose-induced insulin secretion and stimulates hypoglycemia-induced glucagon secretion through activation of the SNS (31). Recently, Monokoshi et al. (101) provided convincing evidence that leptin directly stimulate AMPK activity and increases fatty oxidation in skeletal muscle as well as via the SNS. They demonstrated that an intravenous injection of leptin increases AMPK activity in the skeletal muscle at 15 min and at 6 hours after injection. To determine whether the effects of leptin on AMPK activation require the hypothalamic-sympathetic nervous system axis, they used pharmacological adrenergic blockade and two kinds of surgical denervation. Denervation blocked the ability of intrahypothalamic leptin or intravenous leptin 6 hours after injection to stimulate alpha 2 AMPK in soleus muscle. However, alpha2 AMPK activation at 15 min after i.v. leptin remained intact in denervated muscle. This result suggests that leptin exerts its effect directly to the skeletal muscle within 15 min from the intravenous leptin injection, whereas, it takes three to six hours for SNS mediated leptin effect to increase AMPK activity in the skeletal muscle.

Tang-Christensen et al. (34) reported that leptin administration increases circulating norepinephrine levels by 55% in monkeys. Also, Satoh et al. reported increased norepinephrine and epinephrine levels in a dose -dependent manner after a leptin injection. Increase in catecholamine levels would increase RMR. The literature also suggested that the activity of the SNS was a determinant of energy expenditure and that individuals with low resting SNS may be at risk for body weight gain because of the lower metabolic rate (23-26). Therefore, it is likely that leptin increases overall sympathetic nerve activity, thereby leading to a significant increase in energy expenditure.

Non-SNS related regulating factors for circulating leptin

Regulation of leptin expression by nutrition is probably mediated in part by insulin (70-73). Insulin directly regulates *ob* gene expression and changes in plasma insulin levels under different physiological states have resulted in positively correlated changes in leptin concentrations (71). The changes in leptin levels during euglycemic clamp tests depend on the rate of insulin infusion, indicating a dose-dependent

stimulatory effect of insulin on leptin secretion (70). These studies suggest that insulin stimulates leptin production and release, thereby contributing to the regulation of fasting- and meal- induced modulation of leptin levels.

Glucose has also been shown to influence leptin levels (102-103). Mueller et al. (102) demonstrated that glucose metabolism influences leptin release from cultured adipocytes, suggesting that circulating leptin may be regulated by insulin- and glucose-mediated changes in glucose utilization. Maintaining euglycemia by continuous low-dose glucose infusion during 72h of fasting prevents the normal decline in plasma leptin concentration, suggests that plasma glucose availability regulates plasma leptin levels (103).

Leptin levels are also regulated by other hormonal factors (104-108). Glucocorticoids directly stimulate leptin synthesis in cultured adipocytes, and leptin expression increases in response to chronic elevation of cortisol in humans (104). Tan et al. (104) reported that short-term dexamethasone treatment increases leptin gene expression and secretion about 2-fold and 1.4 fold respectively. They also reported that dexamethasone-induced plasma leptin increase was independent of circulating insulin. However, it is not clear whether dexamethasone stimulates leptin production or causes central or peripheral leptin resistance. TNF-α also increases leptin production and this occurs within 12 h in vivo (105-106). In addition, thiazolidines, which stimulate peroxisome proliferator-activated receptor-γ a regulator of adipose tissue differentiation, decrease leptin production (107).

IMPAIRED SYMPATHETIC NERVOUS SYSTEM IN PEOPLE WITH SCI

Anatomical and physiological changes after SCI

Complete spinal cord lesions result in pathophysiological changes, including: 1) loss of supraspinal regulatory control (18-20, 108); 2) reduced sympathetic activity (21, 109); and 3) morphologic changes in the sympathetic preganglionic neurons (27). As a result, hormonal and metabolic responses to metabolic challenges are altered in people with SCI, especially in people with cervical and high level thoracic SCI (high lesion SCI) (110-114). Several research studies have identified the area of the 6th thoracic spinal cord segment (T6) as the critical site for sympathetic innervation of adrenal medulla (18, 20).

Individuals with SCI below T6 demonstrate sympathoadrenal activity similar to the AB population (20). However, individuals with high lesion SCI experience diminished sympathetic nerve activity below the lesion level and a lower whole body norepinephrine turnover, as well as an impaired release of epinephrine from the adrenal medulla (6, 20). Consequently, in people with high lesion SCI, there is no increase in plasma epinephrine concentration during exercise and insulin-induced hypoglycemia when compared to low-level paraplegic subjects or AB subjects (20, 110, 114).

Hormonal and metabolic response to fasting in people with SCI

Dysfunction of the SNS associated with people with high lesion SCI causes changes in hormonal response during fasting (110, 114)). Palmer et al. (114) investigated the catecholamine response to insulin-induced hypoglycemia in AB controls and in subjects with high lesion SCI and AB subjects. They found that catecholamine levels did not increase in people with high lesion SCI while it increased more than 200% in AB controls. This result was confirmed by another study (28), in which a 12.8 fold increase in norepinephrine during insulin-induced hypoglycemia in AB controls was reported, while no changes were observed in the high lesion SCI group. Although the afferent limb of the baroreceptor arc is intact in people with high lesion SCI, the sympathetic component of the efferent limb is disrupted. This results in an inability to increase sympathetic nervous activity. Interestingly, Corrall et al. (110) reported that people with high lesion SCI have not lost the ability to restore normoglycemia after insulin- induced hypoglycemia, suggesting that the SNS is not the only system involved in restoring blood glucose during hypoglycemia.

Hormonal and metabolic response to exercise in people with SCI

The SNS plays an important role in the regulation of the metabolic and neuroendocrine response to exercise (20, 78, 79, 115). During graded exercise tests in the AB population, stimulation of the peripheral sympathetic system from the CNS is observed, resulting in an exponential increase in free plasma epinephrine and norepinephrine (78, 79). However, people with high lesion SCI have a blunted catecholamine response to incremental exercise (18, 20). Catecholamine and lactate

responses to an incremental VO2 peak test were measured in three men with SCI above T6 and four men with SCI below T6. Subjects with a lower level injury showed a higher epinephrine (Low vs High: 0.5±0.2 vs 0.1 ng/ml), norepinephrine (3.1±1.0 vs 0.48±0.11 ng/ml) and lactate (7.1±0.5vs 3.2±0.4 mM) response compared to subjects with a higher-level injury (18). Schmid et al. (20) also investigated the influence of different injury levels on epinephrine and norepinephrine at rest and during graded wheelchair exercise. People with a SCI above C7 showed a lower epinephrine and norepinephrine level at rest and only a slight increase during exercise compared to the other subject group whose injury level was lower.

Plasma catecholamines exert potent effects on glucose metabolism by stimulating lipolysis in adipose tissue, thus inhibiting excessive glucose consumption (115). Plasma catecholamines contribute greatly to the maintenance of glucose homeostasis during and after exercise (78, 79). Norepinephrine release at the nerve endings of the islets of Langerhans diminishes insulin release during exercise, thereby enhancing FFA mobilization (69). In contrast, people with high lesion SCI have an impaired ability to increase norepinephrine release at the nerve endings of the β-cells in response to exercise (111-112). Therefore, a SNS abnormality leads to different hormonal and metabolic responses to exercise (111-112). Kjaer et al. (111) measured glucose turnover and other hormonal responses to exercise using functional electrical stimulation (FES) and voluntary arm crank at the same VO2 in subjects with high lesion SCI and compared the results with an AB control group. They found decreased glucose levels only in the SCI group due to unchanged glucose production and increased glucose uptake. In the normal condition, however, aerobic exercise maintains glucose levels since glucose uptake is compensated by increased hepatic glucose production and decreased insulin level.

LEPTIN METABOLISM IN PEOPLE WITH SCI

We recently demonstrated that people with high lesion SCI (above C7) had significantly higher plasma leptin levels compared with the AB group (4, 21). These findings are consistent with other studies (116, 117). Bauman et al. (116) investigated the relationship between plasma leptin levels, BMI and percent body fat in 48 AB male

controls and 34 male subjects with SCI. They reported that plasma leptin was significantly higher in the SCI group compared to the control group (12.7± 1.7 vs 7.6 ± ng/ml, $p<0.005$). This result is in agreement with the study done by Huang et al. (117) who reported significantly higher plasma leptin levels among 47 men with SCI compared to the AB controls (6.23±0.66 vs 3.07±0.31 ng/ml, $p<0.001$). Adiposity appears to predict plasma leptin levels in people with high lesion SCI as well as in the general population. In our study we found increased percent body fat in the SCI group compared with the AB group. However, when fat mass was controlled (plasma leptin per kilogram of fat mass), leptin levels remained 45% higher in the SCI group compared with the AB group. Although this difference was not statistically significant, these findings suggest that factors other than fat mass may also be responsible for the higher leptin levels in this group. Recent evidence suggests that adrenergic activation may modulate leptin levels. Pinkney et al. (67) demonstrated that infusion of a ß-adrenergic agonist (isoprenaline) rapidly reduces circulating levels of leptin. As well, Commins *et al.* (68) illustrated that leptin's effect on gene expression in brown and white adipose tissue is dependent upon norepinephrine synthesis and secretion. These results suggest the presence of a tonic inhibitory adrenergic influence on leptin secretion. Removal of this inhibition would probably contribute to increased plasma leptin levels. Our data support reduced SNS activity accompanied by significantly lower norepinephrine levels in people with SCI subjects (4).

In addition, we found that leptin does not correlate with RMR in the SCI group, while leptin highly correlated with RMR in AB group (21). This result provides the evidence that normal SNS function is required for leptin's influence on energy expenditure. Knowing that one of leptin's roles is to maintain body weight homeostasis by regulating RMR (32-34), obesity in people with SCI may be due to failure of SNS-mediated RMR regulation (21). SNS ß-adrenergic support of RMR in the healthy population accounts for approximately 71 kcal/d. In other words, those with a reduced sympathetic tone would need to compensate 26,000 kcal/yr through decreased energy intake or increased energy expenditure by non-SNS mechanisms to prevent weight gain.

We also demonstrated that leptin's response to fasting is altered in people with SCI (4). A fasting-induced decrease in plasma leptin plays an important role in initiating

changes in substrate metabolism and energy expenditure (39, 40). We demonstrated that plasma leptin decreased with 36 h of fasting by 48.8 ± 4.5 and 38.6 ± 7.9% in both the SCI and the AB groups, respectively. The literature in this area suggests that short-term fasting (12 h) reduces plasma leptin levels in the AB population (46-50). Our data are consistent with these findings (4). Plasma leptin was reduced significantly after 12 h of fasting in the AB group but was not altered until 24 h in the SCI group. The mechanism for the delayed leptin response in the SCI group may be related to SNS dysfunction and adiposity level. Leptin mediates many of its physiological effects by activating neurons in the hypothalamus that innervate and stimulate sympathetic preganglionic neurons in the spinal cord (118-119). In individuals with high-lesion SCI, these neurons are atrophied (27), and stimulation below the lesion may be impaired (6, 18, 19, 20). Consequently, impairment of the SNS may alter leptin secretion and as well as its metabolic effects in people with SCI (4, 21).

EFFECTS OF EXERCISE TRAINING ON TYPE 2 DM.

Increase in fat mass and changes in skeletal muscle biochemical and histochemical characteristics are the two main causes of insulin resistance in people with SCI (6-7, 10-17). Body composition changes abruptly in a few years after SCI due to decreased physical activity as well as impaired SNS (4-7). Increased fat mass is one of the risk factors for insulin resistance as mentioned earlier in this paper. Also, skeletal muscle below the level of an upper motor neuron lesion undergo marked changes in morphological, metabolic and contractile properties following SCI (120-123). Skeletal muscle changes in people with SCI include: 1) a pronounced reduction, or complete disappearance of Type I fibres and Type IIa fibres (120, 122), 2) reduced capillary density and blood flow in paralyzed muscles (124 125), 3) reduced content of GLUT-4 in paralyzed muscle (121, 126), 4) activity of oxidative enzymes (121, 127)

Interestingly, the proportion of muscle fibre Type I and IIa, capillarization, GLUT-4 content, oxidative enzymes appear to be correlated with insulin sensitivity (16, 121, 129). This was demonstrated by Lillioja et al. (129) who compared the capillary density and muscle fibre types in the vastus lateralis with in vivo insulin sensitivity determined by the euglycemic clamp. They found that insulin sensitivity was correlated

with percent Type I fibres (r=0.29) as well as capillary density (r=0.63) and negatively correlated with percent Type II b fibres (r=-0.38). Also, the maximal capacity for insulin to stimulate glucose transport was positively correlated with the total GLUT-4 content of muscle (127). Therefore, it is suggested that changes in muscle characteristics after paralysis are one of the major risk factors for insulin resistance in people with SCI.

Can these SCI-induced changes in muscle characteristics be reversed to pre-injury levels and eventually restore normal insulin sensitivity with exercise training? Very few studies have examined the effects of exercise training on insulin sensitivity, body composition and hormone levels in people with SCI (126, 128, 130, 131, 132). We have examined the effects of eight weeks of FES Cycling (3 days/week, 30 minutes/day) on GLUT-1, GLUT-4, capillary number, size of fiber area, glucose tolerance and insulin sensitivity in people with SCI (126, 130). We demonstrated that eight weeks of training with FES cycling improved insulin sensitivity and glucose tolerance in people with SCI, related to a 72% increase in GLUT-4 content and a 52% increase in GLUT-1 content. Hjeltnes et al. (128) reported a 378% increase in GLUT-4 content, glycogen synthase (526±146%) and hexokinase II (204±47%) in vastus lateralis muscle in people with SCI after daily training for eight weeks with FES cycling. They also reported increased whole body insulin-stimulated glucose uptake by 33±13% and 2.1-fold increase in insulin-stimulated (100 microU/ml) 3-O-methylglucose transport in isolated vastus lateralis muscle. In addition, Mohr et al. (132) also examine the effect of one year of FES cycling on insulin sensitivity and GLUT-4 in individuals with SCI. Whole body insulin sensitivity was increased but reduction of training from three times per week to once per week failed to maintain-training induced improved insulin sensitivity. Anderson et al. (120) demonstrated that 12 months of exercise training with FES cycling decreased Type IIb from 37.2% to 2.3%, Type IIb/IIa from 40.7% to 4.6%, while the number of Type IIa increased to 91.2% from 21.2% before training. Therefore, exercise with FES cycling is capable of restoring SCI-induced changes in muscle characteristics and improve insulin sensitivity in people with SCI.

One of the reasons for the high risk of developing Type 2 DM in people with SCI is increased fat mass. Hjeltnes et al. (131) reported that eight weeks of exercise training with FES cycling decreases fat mass (29.7±2.6 to 27.8±2,1%, $p<0.05$) and increases lean

body mass (66.2±2.6 to 68.2±2.1%, $p<0.05$). We have also recently determined the effects of exercise training with FES-rowing on body composition in people with SCI (5). We found a 7.5% and 8.8% reduction in total body fat mass and trunk fat mass, respectively after 12 weeks of training with FES-rowing (5). Reduced fat mass especially abdominal fat decreases the risk of developing Type 2 DM and cardiovascular disease. Therefore, exercise-induced reduction in fat mass would decrease the risk of developing Type 2 DM.

SUMMARY AND RECOMMENDATION

In summary, people with SCI are more prone to obesity and obesity-related disorders and this may be associated with an impaired SNS and decreased physical activity. Leptin, known as the anti-obesity hormone, requires an intact SNS function for its normal regulation and metabolic effects. People with SCI have impaired SNS and therefore, the risk of developing obesity and obesity-related disorders may increase in this population. Exercise is one of the best ways to prevent and treat obesity and obesity-related disorders. Although we have observed positive effects of exercise on body fat and insulin sensitivity, effects of exercise on plasma leptin or leptin's metabolic effects have not been investigated in people with SCI.

REFERENCES

1. Bauman WA, Spungen AM. 2000 Metabolic changes in persons after spinal cord injury. Physical Med Rehab Med Clin North Am 11:109-140.
2. Bauman WA, Spungen AM. 1994. Disorder of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: A model of premature aging. Metabolism. 43:749-756.
3. Duckworth WC, Solomon SS, Jallepall P. 1983 Glucose intolerance in spinal cord injury. Arch Phy Med Rehab. 64: 107-110.
4. Jeon YJ, Harber VJ, Steadward RD. (2002) Leptin response to short term fasting in sympathectomized men- role of sympathetic nervous system. American Journal of Physiology: Endocrinology and Metabolism 284:E634-E640.
5. Jeon YJ, Hittinga D, Suh MY, Steadward RD, Wheeler GD. 2003 Effects of 12 weeks of FES-Rowing training on insulin sensitivity and body composition. (Unpublished data).
6. Karlsson AK, Elam M, Friberg P, Sorensen FB, Sullivan L, Lonnroth P. 1997 Regulation of lipolysis by the sympathetic nervous system: A microdialysis study in normal and spinal cord injured subjects. Metabolism. 46:388-394.
7. Olle MM, Pivarnik JM, Klish WJ, Morrow JR. 1993. Body composition of sedentary and physically active spinal cord injured individuals estimated from total body electrical conductivity. Arc Phy Med Rehab 74:706-710.
8. Bjorntorp P, 1990 Portal adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 10:439-496.
9. Kadowaki T. 2000 Insight into insulin resistance and type 2 diabetes from knockout mouse models. J Clin Invest 106:459-465.
10. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, et al. 1997 Body fat distribution and risk of noninsulin-dependent diabetes mellitus in women. The Nurse's Health Study. Am J Epidemiol. 145:614-619.
11. Hatamisligil GS, Spiegelman BM. 1994 Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes. 43: 1271-1278.

12. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. 2001 Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol. 280:E745-E751.

13. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. 1996 The expression of TNF alpha by human muscle: relationship to insulin resistance. J Clin Invest. 97:1111-1116.

14. Dresner A, Laurent D, Marcucci M, et al. 1999 Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinostitol 3-Kinase activity. J Clin Invest. 103:253-259.

15. Roden M, Price TB, Perseghin G, et al. 2001 Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest. 97:2859-2865.

16. Kahn BB. 1992 Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. J Clin Invest 89:1367-74.

17. Zierath JR, Housenecht KL, Gnudi L, Kahn BB. 1997 High-fat feeding impairs insulin-stimulated GLUT-4 recruitment via an early insulin-signaling defect. Diabetes. 46:215-22.

18. Frey GC, McCubbin JA, Dunn JM, Mazzeo RS. 1996 Plasma catecholamine and lactate relationship during graded exercise in men with spinal cord injury. Med Sci Sports Exerc. 29:451-456.

19. Lehmann KG, Shandling AH, Yusi AU, Froelicher VF. 1989 Altered ventricular repolarization in central sympathetic dysfunction associated with spinal cord injury. Am J Cardiol. 63:1498-1504.

20. Schmid A, Huonker M, Barturen JM, Stahl F, Schmidt-Trucksass A. 1998 Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. J Appl Physiol. 85:635-641.

21. Jeon YJ, Harber VJ, Bell G, McCargar L, Steadward RD, Wheeler GD. (2003) Intact Sympathetic Nervous System is Required for Leptin Effects on Resting Metabolic Rate in People with Spinal Cord Injury. J Clin Endocri Metab 88:402-407.

22. Monroe MB, Tataranni PA, Pratlley R et al. 1998 Lower daily energy expenditure as measured by a respiratory chamber in subjects with spinal cord injury compared with control subjects. Am J Clin Nutr. 68:1223-1227.

23. Peterson HR, Rothchild M, Weinsberg CR, Fell RD, McLeish KR, Pfeifer MA. 1988 Body fat and the activity of the autonomic nervous system. N Engl J Med. 318:1077-1083.

24. Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E et al. 1993 Body fat and sympathetic nerve activity in healthy subjects. Circulation. 89:2634-2640.

25. Schwartz MW, Seeley R, Campfield A, Burn P, Baskin DG. 1996 Identification of Targets of leptin action in rat hypothalamus. 98:1101-1106.

26. Spraul M, Ravussin E, Fontvieille M et al. 1993 Reduced sympathetic nervous activity- A potential mechanism predisposing to body weight gain. J Clin Invest 92:1730-1735.

27. Krassioukov AV, Bunge RP, Pucket WR, Bygrave MA. 1999 The changes in human spinal sympathetic preganglionic neurons after spinal cord injury. Spinal Cord. 37:6-13.

28. Mathias CJ, Frankel HL, Turner RC, and Christensen NJ. Physiological responses to insulin hypoglycemia in spinal man. *Paraplegia* 17: 319-326, 1979-1980.

29. Haque MS, Minokoshi Y, Hamai M, Iwai H, Horiuchi M, Shimazu T. 1999 Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. Diabetes. 48:176-1712.

30. Minokoshi Y, Haque MS, Shimazu T. 1999 Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes. 48:287-291.

31. Mizuno A, Murakami T, Otani S, Muwajima M, Shima K. 1998 Leptin affects pancreatic endocrine function through the sympathetic nervous system. Endorinology. 139:3863-3870.

32. Satoh N, Ebihara K, Ogawa Y, Masuzaki H, Katsuura G. 1999 Sympathetic activation of leptin via the ventromedial hypothalamus-leptin induced increase in catecholamine secretion. Diabetes. 48:1787-1793.

33. Scarpace PJ, Matheny M, Pollock BH, Tumer N.1997 Leptin increase uncoupling protein expression and energy expenditure. Am J Physiol. 273: E226-E230.

34. Tang-Christiansen M, Havel PJ, Jacobs R et al. 1999 Central administration of leptin inhibit food intake and activates the sympathetic nervous system in Rhesus Macaques. J Clin Endocrinol Metab. 84: 711-717.

35. Harvey G. 1969 Regulation of energy balance. Nature 222:629-631.

36. Kennedy G. 1953 The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Sco London 140:578-592.

37. Chen G, Koyama K, Yuan X, et al. 1996 Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proc Natl Acad Sci USA. 93:14795-14799.

38. Zhang Y, Proenca R, Maffei M et al. 1994 Positioning cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.

39. Ahima RS. 1996 Role of leptin in the neuroendocrine response to fasting. Nature 382: 250-252.

40. Ahima RS, Flier JS. 2000 Leptin. Annu Rev Physiol. 62:413-437.

41. Halaas JL, Gajiwala KS, Meffei M, Cohen SL, Chait BT. 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546.

42. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetam CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. 1999 Effets of recombinant leptin theraphy in a child with congenital leptin deficiency. New Eng J Med 341:879-884.

43. Yaspelkis BB, Ansari L, Ramey E, Holland GJ, Loy SF. 1999 Chronic leptin administration increases insulin mediated skeletal muscle glucose uptake and transport. Metabolism. 48:671-676.

44. Considine RV. Sinha MK. Heiman ML. Kriaugiunas A. & Stephens TW. et al. 1996 Serum immunoreactive-Leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334:292-295.

45. Mantzoros CS. Moschos S, Avramopoulos I, Kaklamani V, Liolios A, Doulgerakis DE, Griveas I, Katsilambros N, Flier JS. 1997 Leptin concentrations in relation to body mass index and the tumor necrosis factors-α system in humans. J Clin Endocrinol Metab 82:3408-3413.

46. Boden G, Chen X, Mozzoli M, Ryan I. 1996 Effect of fasting on serum leptin in normal human subjects. J Clin Endo Met 81:3419-3423.

47. Maccario M, Aimaretti G, Gorneli G, Gauna C, Grottoli S. et al. 2000 Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans. Am J physiol Endocrinol Metab 279: E411-E416.

48. Weiss BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R, Gougeon R. 1999 Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentration in obese women. Am J Clin Nutri. 70:321-330.

49. Klein S. Horowitz JF. Landt M. Goodrick SJ & Mohamed-Ali V. et al. 2000 Leptin production during early starvation in lean and obese women. *Am J physiol Endocrinol Metab.* 278: E280-E284.

50. Sonnenberg GE, Krakower GR, Hoffmann RG, Maas DL, Hennes MMI, Kissebah AH. 2001 Plasma leptin concentration during extended fasting and graded glucose infusions: Relationships with changes in glucose, insulin and FFA. J Clin Endo Metabol. 86: 4895-4900.

51. Duclos M, Corcuff JB, Ruffie A. et al. 1999 Rapid leptin decrease in immediate post-exercise recovery. Clin Endocrinol 50:337-342.

52. Karamouzis I, Karamouzis M, Vrabas IS, Christoulas K, Kyriazis N, Giannoulis E, Mandroukas K. 2002 The effects of marathon swimming on serum leptin and plasma neuropeptide Y levels. Clin Chem Lab Med 40:132-136.

53. Landt M, Lawson GM, Gelgeson JM et al. 1997 Prolonged exercise decreases serum leptin concentration. Metabolism 46:1109-1112.

54. Fisher JS, Van Pelt RE, Zinder O. et al 2001 Acute exercise effect on postabsorptive serum leptin. J Appl Physiol. 91:680-686.

55. Dirlewanger M, Vetta VD, Giusti V, Schneiter P, Jequier E. et al. 1999 Effect of moderate physical activity on plasma leptin concentration in humans. Eup J Appl Physiol. 79:331-335.

56. Hilton LK, Loucks AB. 2000 Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am J Physiol. 278: E43-E49.

57. Kraemer RR, Johnson LG, Haltom R, Kraemer GR, Hebert EP. et al. 1999 Serum leptin concentrations in response to acute exercise in postmenopausal women with and without hormone replacement therapy. PSEBM 221:171-177.

58. Perusse L, Collier G, Gagnon J. et al. 1997 Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol. 83:5-10.

59. Hickey MS, Considine RV, Israel RG, Mahar TL, McCammon MR. et al. 1996 Leptin is related to body fat content in male distance runners. Am J Physiol: E938-E940.

60. Racette SB, Coppack SW, Landt M, Klein S. 1997 Leptin production during moderate-intensity aerobic exercise. J Clin Endocrinol Metab. 82:2275-2277.

61. Leal-Cerro A, Garcia-Luna RP, Astorga R, Parejo J, Peino R. et al. 1998 Serum leptin levels in male marathon athletes before and after the marathon run. J Clin Endocrinol Metab. 83:2376-2379.

62. Tuominen JA, Ebeling P, Laquier FW, Heiman ML, Stephens T. et al. 1997 Serum leptin concentration and fuel homeostasis in healthy man. Eup J Clin Invest. 27:206-211.

63. Van Aggel-Leijssen DPC, Van Baak MA, Tenenbaum R, Campfield LA, Saris WHM. 1999 Regulation of average 24h human plasma leptin level; the influence of exercise and physiological changes in energy balance. Int J Obes Relat Metab Disord. 23:151-8.

64. Marliss EB, Simantirakis E, Miles PDG, Hunt R, Gougeon-Reyburn R et al. 1992. Glucose turnover and its regulation during intense exercise and recovery in normal male subjects. Clin Invest Med. 15:406-419.

65. Sotsky MJ, Shilo S, Shamoon H. 1989 Regulation of counterregulatory hormone secretion in man during exercise and hypoglycemia. J Clin Endocrinol Metabol. 68:9-16.

66. Murakami T,Iida M, Shima K. 1995 Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem Biophys Res Commun. 214:127-127.

67. Pinkney JH, Coppack SW, Mohamed-Ali V. 1998 Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol 48:407-411.

68. Commins S, Marsh D, Thomas S et al. 1999 Norepinephrine is required for leptin effects on gene expression in brown and white adipose tissue. Endocrinology. 140: 4772-4778.

69. Natali A, Gastaldelli A, Galvan AQ, Sironi AM, Ciociaro D. et al. 1998 Effects of acute β_2-blockade on insulin action and secretion in humans. Am J Physiol 274:E57-E64.

70. Boden G, Chen X, Kolaczynski JW, Polansky M. 1997 Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest 100:1107-1113.

71. Doucet E, ST-Pierre S, Almeras N, Mauriege P, Despres JP, et al. 2000 Fasting insulin levels influence plasma leptin levels independently from the contribution of adiposity: Evidence from both a cross-sectional and an intervention study. J Clin Endocrinol Metab. 85:4231-4237.

72. Getty TW, Harkness PJ, Watson PM. 1996 The β_3 adrenergic receptor inhibits insulin-stimulated leptin secretion form isolated rat adipocytes. Endocrinology 137: 4054-4057.

73. Kolaczynski JW, Nyce MR, Considine RV et al. 1996 Acute and chronic effects of insulin on leptin production in humans: studies on vivo and in vitro. Diabetes 45:699-701.

74. Essig DA, Alderson NL, Ferguson MA. et al. 2001 Delayed effects of exercise on the plasma leptin concentration. Metabolism 49:359-399.

75. Bramlett SB, Zhou J, Harris RBS, Handry SL, Witt TL, Zachwieja JJ. 1999 Does beta3-adrenoreceptor blockade attenuate acute exercise-induced reductions in leptin mRNA? J Appl Physiol 87:1678-1683.

76. Friedman JE, Ferrara CM, Aulak KS, Hatzoglou M, McCune SA. et al. 1997 Exercise training down-regulate ob gene expression in the genetically obese SHHF/Mcc-fa Rat. Horm Metab Res 29:214-219.

77. Zheng D, Wooter MH, Zhou Q, Dohm GL. 1996 The effect of exercise on ob gene expression. Biochem Biophysical Res Commun 225:747-750.

78. Howlett K, Febbraio M, Hargreaves M. 1999 Glucose production during strenuous exercise in humans: role of epinephrine. Am J Physiol. 276:E1130-1135.

79. Mora-Rodriguez R, Coyle EF 2000 Effects of plasma epinephrine on fat metabolism during exercise: interactions with exercise intensity. Am J Physiol, 278:E669-E676.

80. Pagano C, Marzolo M, Granzotto M, Ricquier D, Federspil G, Vettor R. 1999 Acute effects of exercise on circulating leptin in lean and genetically obese fa/fa rats. Biochem Biophysical Res Commu 255, 698-702.

81. Kowalska I, Straczkowski M, Gorski J, Kinalska I. 1999 The effect of fasting and physical exercise on plasma leptin concentrations in high-fat fed rats. J Physiol Pharmacol. 50:309-320.

82. Fogteloo AJ, Meinders AE, PIJL H, Kroon AA, Frolich M, De Leeuw PW. 2001 Renal clearance of endogenous leptin in hypertensive human with or without renal artery stenosis. Am J Physiol Endocrinol Metab. 281: E400-E404.

83.

84. Gutin B, Ramsey L, Barbeau P, Cannady W, Ferguson M, Litaker M, Owens S. 1999 Plasma leptin concentrations in obese children: changes during 4-mo periods with and without physical training. Am J Clin Nutri 69:388-394.

85. Halle M, Berg A, Garwers U, Grathwohl D, Knisel W. et al. 1999 Concurrent reductions of serum leptin and lipids during weight loss in obese men with type II diabetes. Am J Physiol. 277:E277-E282.

86. Kohrt WM, Landt M, Birge SJ. 1996 Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older females. J Clin Endocrinol Metab 81:3980-3985.

87. Okazaki T, Himeno E, Nanri H. et al. 1999 Effects of mild aerobic exercise and a mild hypocaloric diet on plasma leptin in sedentary women. Clin Exp Pharm Physiol 26:415-420.

88. Pasman WJ, Sesterterp-Plantenga MS, Saris WHM. 1998 The effect of exercise training on leptin levels in obese males. Am J Physiol. 274:E280-E286.

89. Ishii T, Yamakita T, Yamagami K, Yamamoto T, Miyamoto M, Kawasaki K, Hosoi M, Yoshioka K, Sata T, Tanaka S, Fujii S. 2001 Effect of exercise training on serum leptin levels in type 2 diabetic patients. Metabolism 50:1136-1140.

90. Thong FS, Hudson R, Ross R, Janssen I, Graham TE. 2000 Plasma leptin in moderately obese men: independent effects of weight loss and aerobic exercise Am J Physiol. 279: E307-E313.

91. Hickey MS, Houmard JA, Considine RV, Tyndall GL, Midgette JB, Gavigan KE, Weidner ML, McCammon MR, Israel RG, Caro JF 1997 Gender-dependent effects of exercise training on serum leptin levels in humans. Am J Physiol Endocrinol Metab. 272:E562-566.

92. Kreamer RR, Kreamer GR, Acevedo EO, Hebert EP, Temple E, Bates M, Etie A, Haltom R, Quinn S, Castracane VD. 1999 Effects of aerobic exercise on serum leptin levels in obese women. Eur J Appl Physiol 80:154-158.

93. Horowitz JF, and Klein S. 2000 Whole body and abdominal lipolytic sensitivity to epinephrine is suppressed in upper body obese women. *Am J physiol Endocrinol Metab* 278:E1144-E1152.

94. Klein S, Sakurai Y, Romijn JA, and Carroll RM. Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. *Am J Physiol Endocrinol Metab* 265: E801-E806, 1993

95. Carulli L, Ferrari S, Bertolini M, Tagliafico E, Rio G. 1999 Regulation of ob gene expression: Evidence for epinephrine-induced suppression in human obesity. J Clin Endocrinol Metab. 84: 3309-3312.

96. Evan BA, Agar L, Summers RJ 1999 The role of the sympathetic nervous system in the regulation of leptin synthesis in C57BL/6 mice. FEBS Lett 444:149-154.

97. Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS. 1996 Role of leptin in fat regulation. Nature 380:677.

98. Dunbar JC, Hu Y, Lu H. 1997 Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. Diabetes 46:2040-2043.

99. Sivitz WI, Fink BD, Morgan DA. et al. 1999 Sympathetic inhibition, leptin, and uncoupling protein subtype expression in normal fasting rats. Am J Physiol. 277:E668-E677.

100. Rayner DV, Simon E, Duncan JS, Trayhurn P. 1998 Hyperleptinaemia in mice induced by administration of the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine. FEBS letter 429:395-398.

101. Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Muller C, Carling D, Kahn BB. 2002 Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415:339-343.

102. Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, et al. 1998 Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology. 139:551-558.

103. Wellhoener P, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W. et al. 2000 Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. J Clin Endocrinol Metab. 85:1267-1271.

104. Tan JT, Patel BK, Kaplan LM, Keonig JI, Hooi SC. 1998 Regulation of leptin expression and secretion by corticosteroids and insulin-Implication for body weight. 8:85-92.

105. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak Nt et al. 1997 Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med 185:171-175.

106. Grunfeld C, Zhao C, Fuller J, Pollack A, Moser A, et al. 1996 Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. J Clin Invest. 97:2152-2157.

107. Kallen CB, Lazar MA. 1996. Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. Proc Natl Acad Sci. USA 93:5793-5796.

108. Monroe M, Seals D, Shapiro L et al. 2001 Direct evidence for tonic sympathetic nervous support of resting metabolic rate in healthy adult humans. Am J Physiol Endocrinol Metab. 280: E240-744.

109. Debarge G, Christensen NJ, Corbett JL, 1974 Eidelman BH, Frankel HL. Plasma catecholamines in tetraplegics. Paraplegia 12:44-49.

110. Corrall JM, Frier BM, McClemont JW, Taylor SJ, and Christie NE. 1979-1980 Recovery mechanisms from acute hypoglycemia in complete tetraplegia. *Paraplegia* 17: 314-318.

111. Kjaer M Pollack SF, Mohr T. et al. 1996 Regulation of glucose turnover and hormonal responses during electrical cycling in tetraplegic humans. Am J Physiol 271:R191-R199.

112. Kjaer M, Dela F, Sorensen FB. et al. 2001 Fatty acid kinetics and carbohydrate metabolism during electrical exercise in spinal cord injured human. Am J Physiol. 281:R1492-R1498.

113. Kjaer M, Engfred K, Fernandes A. et al. 1993 Regulation of hepatic glucose production during exercise in humans: role of sympathoadrenergic activity. Am J of Physiol. 265:E275-E283.

114. Parmer JP, Henry DP, Benson JW, Johnson DG, Ensinck JW. 1976 Glucagon response to hypoglycemia in sympathectomized man. *J Clin Invest* 57: 522-525.

115. Coker RH, Krishna MG, Lacy DB, Bracy DP, Wasserman DH. 1997 Role of hepatic α- and β-adrenergic receptor stimulation on hepatic glucose production during heavy exercise. Am J Physiol. 273:E831-E838.

116. Bauman WA, Spungen AM, Zhong YG. Mobbs CV. 1996 Plasma leptin is directly related to body adiposity in subjects with spinal cord injury. Horm Metab Res 28:732-736.

117. Huang TS, Wang YH, Chen SY. 2000 The relation of serum leptin to body mass index and to serum cortisol in men with spinal cord injury. Arch Phys Med Rehabil 81:1582-1586.

118. Elias AN, Pandian MR, Wang L, Suarez E, James N, Wilson AF. 1999 Leptin and IGF-1 levels in unconditioned male volunteers after short-term exercise. Psychoneuroendocrinology 25: 453-461.

119. Elias CF, Aschkenasi C, Lee, C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK. Leptin differentially regulate NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23: 775-786

120. Andersen JL, Mohr T, Biering-Sorensen F, Galbo H, Kjaer M. 1996 Myosin heavy chain isoform transformation in single fibres from m. vastus lateralis in spinal cord injured individuals: effects of long-term functional electrical stimulation. Pflugers Archives-European Journal of Physiology 431:513-518.

121. Block NE, Menick DR, Robinson KA, Buse MG 1991 Effect of denervation on the expression of two glucose transporter isoforms in rat hindlimb muscle. J Clin Invest. 88:1546-1552.

122. Burnham R, Martin T, Stein R, Bell G, MaClean I, Steadward RD. 1997 Skeletal muscle fibre type transformation following spinal cord injury. Spinal Cord 35:86-91.

123. Schmalbruch H, Al-Amood WS, Lewis DM. 1991 Morphology of long-term denervated rat soleus muscle and the effect of chronic electrical stimulation. J of Physiol 441:1076-1083.

124. Chilibeck PD, Jeon JY, Weiss CB, Bell G, Burnham RS. 1999 Histochemical changes in muscle of individuals with spinal cord injury following functional electrical stimulated exercise training. *Spinal Cord* 37:264-268.

125. Mohr T, Andersen JL, Biering-Sorensen F, Galbo H, Bangsbo J, Wagner A, Kjaer M. 1997 Long term adaptation to electrically induced cycle training in severe spinal cord injured individuals. Spinal Cord 35:1-16.

126. Chilibeck PD, Bell G, Jeon JY, Weiss CB, & Burnham RS. 1999 Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. *Metabolism* 48: 1409-1413.

127. Henriksen EJ, Rodnick KJ, Mondon CE. 1991 Effects of denervation or unweighting of GLUT-4 protein in rat soleus muscle. J Appl Physiol 70:2322-2327.

128. Hjeltnes N, Galuska D, Bjorholm M, Aksnes AK, Lannem A, Zierath JR, Wallberg-Henriksson H. 1998 Exercise-induced overexpression of key regulatory proteins involved in glucose uptake and metabolism in tetraplegic persons: molecular mechanism for improved glucose homeostasis. FASEB 12:1701-1712.

129. Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WG, Zawadzki JK Yki-Jarvinen, H, Chrisin L, Bogardus C. 1987 Skeletal muscle capillary density and fibre type are possible determinants of in vivo insulin resistance in men. J Clin Invest 80:415-424.

130. Jeon YJ, Weiss BC, Steadward RD, Ryan E, Burnham R, Bell G, Chilibeck P, Wheeler GD 2002 Improved glucose tolerance and insulin sensitivity after electrical stimulation-assisted cycling in people with spinal cord injury, Spinal Cord 40:110-117

131. Hjeltnes N, Aksnes AK, Birkeland KI, Johansen J, Lannem A, Wallberg-Henriksson H. 1997 Improved body composition after 8 wk of electrically stimulated leg cycling in tetraplegic patients Am J Physiol 273: R1072-R1079.

132. Mohr T, Dela F, Handberg A, Biering-Sorensen F, Galbo H, Kjaer M. 2001. Insulin action and long-term electrically induced training in individuals with spinal cord injuries. Med Sci Sports Exer. 33:1247-1252.

REVIEW PAPER TWO

What are the true effects of exercise on leptin regulation and action?

Are we measuring what we intend to measure?

INTRODUCTION:

The identification of the *ob* gene using positional cloning and the characterization of the hormone leptin have greatly increased the understanding of the pathophysiology of obesity and its related disorders (1-3). Leptin deficiency, as seen in *ob/ob* mice is perceived by the hypothalamus as an energy deficient state, which leads to increased energy intake and decreased energy expenditure, resulting in obesity (2, 4). Conversely, the administration of leptin to *ob/ob* mice and children with congenital leptin deficiency decreases feeding, normalize body fat, body temperature, and also correct neuroendocrine abnormalities (2-5). However, the findings that the leptin levels are elevated in obese humans in proportion to the amount of fat mass, suggests that human obesity is related to leptin resistance rather than leptin deficiency (6). One potential reason for leptin overproduction is a defect in the leptin receptor, such as in *db/db mice*, but genetic leptin receptor defects are unlikely to account for more than a small number leptin resistance in humans (3, 6). Therefore, current research aims to understand the cause of leptin resistance both at the level of hypothalamus and peripheral tissues (7-9).

Since the discovery of leptin, many investigations have been conducted to understand the effects of acute aerobic and chronic exercise on leptin (10-20). However, leptin's response to exercise is varied including decreased (10-14), unchanged (15-19) and increased (20) following exercise. In this review, the effect of acute and chronic aerobic exercise on plasma leptin levels, the mechanism by which acute and chronic aerobic exercise influence plasma leptin levels, and the possibility of changes in leptin sensitivity after exercise will be discussed.

EFFECTS OF AEROBIC EXERCISE ON LEPTIN

Leptin response to acute exercise

Following an acute bout of exercise, plasma leptin levels have been reported to be decreased (10-15), unchanged (16) or increased (17-20). Activities such as 30 –60 min of cycling exercise (17) and a 20-mile treadmill run (18) did not change leptin levels. On the other hand, other studies noted that a 2 h run at 75% VO2 max (15), a marathon run (13) and an ultramarathon run (12) significantly decreased plasma leptin levels. The studies that observe the decreased leptin levels following physical activity often involve

exercise of longer durations at higher intensities, resulting in higher energy expenditure (10-15). Evidence of this comes from Landt et al. (13) who detected a 32% reduction in plasma leptin after a 101mile ultramarathon but not after 2h of strenuous cycling. In addition, Karamouzis et al. (12) reported a 50.5% reduction in leptin levels after marathon swimming (25 km sea swimming, 6.5-10.5 hours). These data suggest that higher exercise energy expenditures resulting in higher negative energy balance are more likely to alter leptin levels.

Although a greater exercise-induced negative energy balance would more likely cause a reduction in plasma leptin, controversial issues remain. Studies which observe decreased leptin levels following exercise either involve extremely high energy expenditure (11-13) or have obtained plasma samples during the 2h - 48h of recovery period (10, 14-15) as opposed to immediately following the exercise bout (16, 19). Following exercise bouts of similar duration and intensity, Duclos et al. (10) detected a 30% reduction in plasma leptin 2 h after the cessation of the exercise but Hickey et al. (19) found no difference when samples were taken immediately following exercise. Additionally, Tuominen et al (15) reported a 34% reduction in plasma leptin levels 44 h after 2h running at 75% VO2max and Essig et al. (24) reported a 30% reduction in plasma leptin levels after 48 h after two treadmill exercise sessions with different energy expenditures (800 or 1,500 kcal) at 70% VO2max.

These discrepancies in findings may be explained by hormonal response to intense and prolonged exercise (21-22). Counterregulatory hormones are critical for the maintenance of glucose homeostasis during exercise (21-22). During intense exercise (above 80% VO2max), hepatic glucose production increases up to 8-fold while plasma catecholamines may increase up to 15-fold (21). Plasma insulin may remain constant or decrease slightly, whereas glucagon and cortisol will increase 2- and 3- fold, respectively (20). During low to moderate intensity exercise, plasma epinephrine and norepinephrine increase to a lesser extent whereas cortisol does not increase substantially (20). Cortisol is known to increase leptin production, while catecholamines are known to reduce plasma leptin production (23-25). Also, a recent investigation demonstrated that the treatment of rat adipocytes with insulin increased tissue leptin content and secretion during a 2 h incubation period (26). Therefore, if samples are

collected during and immediately after exercise bouts, exercise-induced hormonal changes may interfere with each other on leptin production in the adipocytes and we may not observe any changes in plasma leptin levels.

Leptin production vs Leptin clearance

Animal studies generally produce consistent results on the effects of exercise on leptin gene expression (27-30). Zheng et al. (30) reported that a single bout of exercise significantly decreased *ob* mRNA levels approximately 30% immediately and 3 h after exercise in male Sprague-Dawley rats. They also reported that 4 weeks of exercise training decreased the *ob* gene expression by 48% at 2 h after the last bout of exercise training compared to the control group. Bramlett et al. (27) reported that treadmill exercise until exhaustion (average 85.5±1.5 min) decreased leptin mRNA significantly in retroperitoneal fat. Moreover, the decrease in leptin mRNA after exercise resulted from stimulation of cyclic AMP-dependent protein kinase and beta-3 adrenergic receptors. Norepinephrine, as well as specific beta 3 adrenergic agonists, reduce leptin expression in white adipose tissue (31, 32). Intense exercise increases norepinephrine levels up to 14 fold, which in turn increases cyclic AMP-dependent protein kinase necessary to decrease leptin expression (24, 27). Therefore, it can be speculated that the exercise-induced catecholamine increase may reduce leptin gene expression in these animals.

However, animal studies still produce conflicting results on the effects of exercise on plasma leptin levels (27, 29). Pagano et al. (29) reported a 30% reduction in plasma leptin after 30 min swimming exercise. On the other hand, Bramlett et al. (27) reported a 59% increase in circulating leptin level after 55 min of treadmill running. This conflicting result could be related to leptin clearance during exercise. While leptin gene expression solely represents leptin production, circulating leptin levels can be contributed to leptin clearance as well as leptin production. Fogteloo et al. (33) recently reported that 80% of the total body leptin clearance could be attributed to the kidney. Interestingly, intense exercise decreases blood flow to the kidney and thus may decease leptin clearance during intense exercise (34). With the slight decrease leptin production, reduction in leptin clearance may end up increasing circulating plasma leptin levels. This may explain studies that have reported no change in leptin levels immediately after

exercise report a reduction in plasma leptin levels 2 to 44 hours after the cessation of an exercise bout (10, 14, 15). We may speculate that a reduction in renal leptin clearance during exercise may compensate or overcompensate exercise-induced reduction in leptin production. In combination of the result from human and animal studies, it is evident that exercise can decrease leptin mRNA and leptin production in adipose tissue. However, due to the lack of control in leptin clearance and counter-regulatory hormones that affects leptin production during exercise, we may observe conflicting results on the effects of exercise on circulating leptin level.

Leptin response to chronic aerobic exercise

There is also considerable variation in the reported effects of chronic exercise on circulating leptin levels (35-40). Studies have demonstrated a reduction (35-38), or no change (39-40), in plasma leptin following an exercise training program. The differences in these findings could be explained by concurrent weight loss with exercise training. Studies observed weight loss with training have also observed a reduction in plasma leptin levels (35-37). Halle et al. (35) studied plasma leptin levels in 20 men with type 2 DM before and after six weeks of training (2,200kcal/wk). They reported that their exercise program caused a 4.3% weight loss and a 30% reduction in plasma leptin levels. Kohrt et al. (36) also reported a 4.9% loss in body mass and a 27.9% reduction in plasma leptin levels after nine months of training. These results are not unexpected, as leptin is known to be produced and secreted by adipose tissue and it is well documented that plasma leptin correlates with body fat mass (6).

Although there is convincing evidence that exercise training that leads to weight loss will decrease plasma leptin levels, it is unclear whether exercise training-induced leptin reduction is solely dependent on changes in fat mass. Hickey et al. (40) reported a reduction in plasma leptin levels in females after 12 weeks of training (4d/wk, 1200 kcal/wk) despite stable fat mass. Perusse et al. (18) also reported a reduction in plasma leptin in obese males after 10 months of exercise training independent of changes in body fat mass. Furthermore, Okazaki et al. (37) also demonstrated that plasma leptin levels decreased in 14 subjects whose fat mass either did not change or slightly increased after a 9 month exercise intervention. Moreover, Ishii et al. (41) report a reduction in

plasma leptin with the combination of diet and exercise, but not with diet alone after 6 weeks of exercise training. Ishii et al. (41) also standardized leptin level per kg fat mass and report that plasma leptin per kg fat mass was lower in the combination of the diet and exercise group as compared to the diet only group. These studies raise some very interesting questions. First, if exercise changes plasma leptin levels without fat mass loss, what is the mechanism for decreased plasma leptin levels? Secondly, if exercise does not change plasma leptin levels without fat mass change, should we consider that there is no effect of exercise training on leptin action or regulation?

ARE WE MEASURING WHAT WE INTEND TO MEASURE?

Early studies on the effects of aerobic exercise training in Type 2 DM failed to show significant improvements assessed by fasting plasma glucose or insulin level (42). After the glucose clamp technique was developed and widely used, subsequent studies demonstrated beneficial effects of exercise on Type 2 DM due to improvement in insulin sensitivity independent of fasting plasma glucose and insulin changes after exercise training (43-46). The same phenomena may explain conflicting results in the leptin response to acute and chronic exercise. Just as one week of exercise does not change fasting glucose or insulin levels but improves insulin sensitivity (46), so plasma leptin levels do not change after one week of aerobic exercise even though leptin sensitivity may be greatly enhanced.

Evidence of leptin's direct effects in peripheral tissue

There are a total of five spliced forms of leptin receptors: Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd and Ob-Re (3). The receptors share an identical extracellular ligand-binding domain of 840 amino acids at the amino terminus as well as a transmembrane domain of 34 amino acids (3, 47, 48). The Ob-Rb is the only isoform with all the protein motifs: an extracellular domain, capable of binding to leptin; a short transmembrane region; and a cytoplasmic region, capable of signal transduction (3). Leptin mediates its intracellular signaling by a member of the Janus family of protein kinases (JAK), specifically increasing the hypothalamic expression of signal transducers and activators of transcription (STAT)-3, 5 and 6 (3). It has been hypothesized that only functional leptin

receptor is long form (OB-Rb), which was known to be located in hypothalamus. However, recent evidence suggests that peripheral organs including the skeletal muscle and adipose tissue also express OB-Rb (3). This fact raises a question as to whether or not leptin may exert directly at the level of target tissue.

Minokoshi et al. (49) provided convincing evidence that leptin stimulates Adenosine 5'-monophosphate-activated protein kinase (AMPK) activity directly in skeletal muscle as well as via the sympathetic nervous system (SNS). AMPK regulates lipid oxidation in muscle by reducing acetyl-CoA carboxylase (ACC) activity, which in turn decreases malonyl-CoA levels and increases the mitochondrial uptake of free fatty acid (FFA). They demonstrated that an intravenous injection of leptin increases AMPK activity in skeletal muscle at 15 min and at 6 hours after injection. To determine whether the effects of leptin on AMPK activation require the hypothalamic-sympathetic nervous system axis, they used pharmacological adrenergic blockade and two kinds of surgical denervation. Denervation blocked the ability of intrahypothalamic leptin or intravenous leptin 6 hours after injection to stimulate alpha 2 AMPK in the soleus muscle. However, alpha2 AMPK activation at 15 min after intravenous leptin injection remained intact in denervated muscle. This study demonstrated that leptin exerts its effect directly to the skeletal muscle within 15 min from an intravenous leptin injection, whereas, it takes three to six hours for a SNS mediated leptin affect to increase AMPK activity. These findings suggest that leptin has peripheral effects on skeletal muscle by directly stimulating AMPK activity. When we combine the findings of Minokoshi et al. (49) with the studies by Steinberg et al. (7, 8) who reported a convincing evidence of peripheral leptin resistance, it raises the question as to whether or not exercise would change peripheral leptin resistance.

Leptin's direct effects on glucose uptake

Barzilai et al. (50) found that following 8 days of leptin treatment in Spraque-Dawley rats, whole body glucose uptake as assessed by the euglycemic clamp technique was increased 52% compared with control animals. Since over 80% of the glucose load is disposed by skeletal muscle, it is possible that leptin may be capable of acting on skeletal muscle to improve insulin action. Burks et al. (51) demonstrated that mice

lacking insulin receptor substrate (IRS)-2 have five fold increased leptin levels by 8 weeks old and they demonstrated that IRS-2 play an important role in the leptin signaling pathway which involves STAT3 phosphorylation. In addition, Kim et al. (52) recently demonstrated that three minutes after intravenous leptin injection in normal rats, there is an increased phosphorylation of STAT3 and STAT1 in adipose tissue and phosphorylation of mitogen-activated protein kinase (MAPK) in adipose tissue and liver. Also, they found that IRS-1 associated phosphatidylinositol-3-OH kinase (PI3-K) activity in adipose tissue and IRS-2-associated PI3-K activity in liver were moderately increased in leptin injected rats. Moreover, Niswender et al. (53) observed the effect of systemically administered leptin on the activity of PI3-K and STAT3 in male Winstar rats and demonstrated that an infusion of PI3-K inhibitor (LY294002) completely block leptin's effects on food intake. Therefore, the defective activation of IRS, PI3-K and MAPK may reduce the ability of leptin to exert its effects in peripheral tissue (50-54). This may cause peripheral leptin resistance.

Evidence of skeletal muscle leptin resistance for fat oxidation

Skeletal muscle is the major tissue contributing to basic metabolic rate and is also the primary tissue responsible for whole body glucose and fatty acid metabolism. Glucose uptake and fatty acid metabolism are regulated by several hormones, including leptin, insulin and catecholamines. As mentioned earlier, human obesity is characterized by high levels of circulating leptin, which suggests the development of central and/or peripheral leptin resistance (7-9, 24). Steinberg and Dick (9) demonstrated that 4 weeks of high fat feeding (high in saturated and n-6 polyunsaturated fatty acid) significantly decreased the rate of fat oxidation in the skeletal muscle (150 to 20 nmol/g/h) in rat soleus muscle. They speculated that high fat feeding induced-skeletal muscle leptin resistance result from increased intramuscular triglyceride (TG). Steinberg et al (8) also demonstrated that leptin stimulates fatty oxidation in skeletal muscle from lean, but not obese subjects in vitro experiment, which is the evidence of skeletal muscle leptin resistance in human. Intravenous leptin administration increases the activity of AMPK which regulates lipid oxidation in muscle by reducing ACC activity, which in turn decreases malonyl coenzyme A levels and

increases mitochondrial uptake of free fatty acid (FFA). Yespelkis III et al. (9) reported that chronic (12-15 days) leptin administration in high fat fed (3-6 months) rats completely reversed high fat diet induced skeletal muscle insulin resistance. They also demonstrated that leptin administration decreases intramuscular TG concentration which improves skeletal muscle insulin sensitivity. Therefore, it is assumed that obesity causes skeletal muscle leptin resistance.

Can exercise improve peripheral tissue leptin sensitivity?

Interestingly, exercise is known to increase phosphorylation of PI3-K, MAPK, AMPK, and activity of IRS (54-57). Chibalin et al. (54) reported that insulin-stimulated tyrosine phosphorylation of IRS-1 and associated PI3-K activity increased 2.5 and 3.5-fold after 1 and 5 days of exercise. After 1 day of exercise, IRS-2 protein expression increased 2.6 fold and basal and insulin-stimulated IRS-2 associated PI3-K activity increased 2.8 fold and 9 fold, respectively. Treadmill running and muscle contractions induced by electrical stimulation also improve MAPK and AMPK activities, which stimulate translocation of GLUT-4 (55-57). Knowing that leptin increases glucose uptake through insulin dependent as well as independent pathways such as IRS-2 expression, IRS-1 associated PI3-K activity, MAPK and AMPK activities, the exercise induced improvement in these factors could improve effectiveness of leptin in the skeletal muscle (54-57).

In addition, exercise increases FFA binding proteins, mitochondrial density and enhances the rate of FFA oxidation in the skeletal muscle and thus decreases the level of intramuscular TG content. Stephens et al. (58) reported that there is a large, progressive increase in ACCβ phosphorylation during moderate-intensity exercise at 60% VO2peak in humans, which is coupled with the progressive increase in AMPKα2 activity and fat oxidation. Knowing that the increased intramuscular TG content in obese individuals is the cause of skeletal muscle leptin resistance, it could be possible that exercise induced reduction in TG content can improve leptin sensitivity. It is also possible that aerobic exercise training could improve skeletal muscle leptin sensitivity and, consequently decrease plasma leptin levels. If the effects of exercise on plasma leptin and leptin sensitivity follow the pattern of the effects of exercise on a plasma insulin and insulin

sensitivity, measuring plasma leptin levels without peripheral leptin sensitivity may not truly represent the effect of exercise on leptin metabolism. Therefore, subjects from studies that report no change in plasma leptin after exercise training may still have improved peripheral leptin sensitivity.

Implication of improvement in skeletal muscle leptin sensitivity

Improvement in leptin sensitivity would could increase effectiveness of leptin on fatty acid oxidation. Acute leptin administration causes a repartitioning or shunting of fatty acid metabolism toward oxidation and away from esterification in skeletal muscle. Leptin also stimulates the hydrolysis of stored intramuscular TG (8, 9, 59). Hence, improved effectiveness of fatty acid oxidation will further decrease intramuscular TG. Unger (60) proposed and provided evidence that leptin is the principal hormone of liporegulation, maintaining normal intracellular lipid homeostasis despite wide variation in dietary fat, much as insulin maintains tolerance to dietary carbohydrate. On a 60% fat diet, adipocyte fat content of normal rats increases almost 150-fold, whereas that of pancreatic islets, liver, heart, and skeletal muscle rises less than 10-fold. He proposed that this low level of TG deposition in nonadipocytes during the consumption of excess dietary fat is the result of release of leptin in proportion to ingested TG excess being stored in adipocytes. Therefore, if leptin is deficient, or if its target tissues become unresponsive during positive energy balance, steatosis will occur. This leads ultimately to so-called lipotoxicity, dysfunction of nonadipose tissue such as the pancreatic β-cells, and skeletal muscle and may culminate in fatty acid-induced apoptosis (60). The net result of lipotoxic disorders in human is syndrome X. As aerobic exercise is known to decrease TG accumulation in the skeletal muscle, improvement in leptin sensitivity may decrease the risk of lipotoxicity and eventually decrease the risk of developing hyperlipidemia, cardiomyopathy, insulin resistance and Type 2 DM, the familiar components of the metabolic syndrome X.

PERSPECTIVE FOR FUTHER RESEARCH

Although there have been many studies to determine the effects of acute/chronic aerobic/resistance exercise on leptin metabolism, the results have been conflicting and

rather confusing because factors that influence leptin production and clearance varies depending on exercise intensity, duration and also blood sampling time. Future studies need to control and compare different exercise intensities, duration and amount of exercise energy expenditure, as well as more frequent and prolonged sampling time. Since energy availability affects leptin production in a significant way, the energy intake also needs to be tightly controlled. Studies also need to control other metabolic and endocrine factors that influence leptin production and clearance. The use of hormone antagonists might be one way to control specific hormones that regulate leptin production during exercise.

In order to determine the true effects of exercise on leptin metabolism, both plasma leptin as well as central and peripheral leptin sensitivity need to be measured. Recently, a few studies addressed the existence of peripheral leptin resistance caused by high fat feeding. However, the effects of acute or chronic exercise on leptin responsiveness for fatty acid oxidation have not been investigated.

REFERENCE

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994 Positioning cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.
2. Halaas JL, Gajiwala KS, Meffei M, Cohen SL, Chait BT. 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546.
3. Ahima RS, Flier JS. 2000 Leptin. Annu Rev Physiol. 62:413-437.
4. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetam CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S.1999 Effets of recombinant leptin therapy in a child with congenital leptin deficiency. New Eng J Med 341:879-884.
5. Scarpace PJ, Matheny M, Pollock BH, Tumer N.1997 Leptin increase uncoupling protein expression and energy expenditure. Am J Physiol. 273: E226-E230.
6. Considine RV. Sinha MK. Heiman ML. Kriaugiunas A. Stephens TW. Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL. 1996 Serum immunoreactive-Leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334:292-295.
7. Steinberg GR, Dyck DJ. 2000 Development of leptin resistance in rat soleus muscle in response to high-fat diets. Am J Physiol Endocrinol Metab. 279:E1374-E1382.
8. Steinberg GR, Parolin ML, Heigenhauser GJF, Dick DJ. 2002 Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. Am J Physiol Endocrinol Metab 283:E187-E192.
9. Yaspelkis III BB, Davis JR, Saberi M, Smith TL, Jazayeri R, Singh M, Fernandes V, Trevino B, Chinookoswong N, Wang J, Shi ZQ, Levin N. 2001 Leptin administration improve skeletal muscle insulin responsiveness in diet-induced insulin resistance rats. Am J Physiol Endocrinol Metab, 280:E130-E142.
10. Duclos M, Corcuff JB, Ruffie A. Roger P, Manier G. 1999 Rapid leptin decrease in immediate post-exercise recovery. Clin Endocrinol 50:337-342.
11. Karamouzis I, Karamouzis M, Vrabas IS, Christoulas K, Kyriazis N, Giannoulis E, Mandroukas K. 2002 The effects of marathon swimming on serum leptin and plasma neuropeptide Y levels. Clin Chem Lab Med 40:132-136.
12. Landt M, Lawson GM, Helgeson JM, Davila-Roman VG, Ladenson JH, Jaffe AS, Hickner RC. 1997 Prolonged exercise decreases serum leptin concentration. Metabolism 46:1109-1112.

13. Leal-Cerro A, Garcia-Luna RP, Astorga R, Parejo J, Peino R. Peino R, Dieguez C, Casanueva FF. 1998 Serum leptin levels in male marathon athletes before and after the marathon run. J Clin Endocrinol Metab. 83:2376-2379.

14. Essig DA, Alderson NL, Ferguson MA. Bartoli WP, Durstine JL.2001 Delayed effects of exercise on the plasma leptin concentration. Metabolism 49:359-399.

15. Tuominen JA, Ebeling P, Laquier FW, Heiman ML, Stephens T. Koivisto VA. 1997 Serum leptin concentration and fuel homeostasis in healthy man. Eup J Clin Invest. 27:206-211.

16. Dirlewanger M, Vetta VD, Giusti V, Schneiter P, Jequier E. Tappy L. 1999 Effect of moderate physical activity on plasma leptin concentration in humans. Eup J Appl Physiol. 79:331-335.

17. Kraemer RR, Johnson LG, Haltom R, Kraemer GR, Hebert EP. Gimpel T, Castracane VD. 1999 Serum leptin concentrations in response to acute exercise in postmenopausal women with and without hormone replacement therapy. PSEBM 221:171-177.

18. Perusse L, Collier G, Gagnon J. Leon AS, Rao DC, Skinner JS, Wilmore JH, Nadeau A, Zimmet PZ, Bouchard C. 1997 Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol. 83:5-10.

19. Hickey MS, Considine RV, Israel RG, Mahar TL, McCammon MR, Tyndall GL, Houmard JA, Caro JF. 1996 Leptin is related to body fat content in male distance runners. Am J Physiol: E938-E940.

20. Fisher JS, Van Pelt RE, Zinder O. Landt M, Kohrt WM. 2001 Acute exercise effect on postabsorptive serum leptin. J Appl Physiol. 91:680-686.

21. Coker RH, Krishna MG, Lacy DB, Bracy DP, Wasserman DH. 1997 Role of hepatic α- and β-adrenergic receptor stimulation on hepatic glucose production during heavy exercise. Am J Physiol. 273:E831-E838.

22. Howlett K, Febbraio M, Hargreaves M. 1999 Glucose production during strenuous exercise in humans: role of epinephrine. Am J Physiol. 276:E1130-1135.

23. Murakami T,Iida M, Shima K. 1995 Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem Biophys Res Commun. 214:127-127.

24. Solano JM, Jacobson L 1999 Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am J Physiol Endocrinol Metab 277:E708-E716.

25. Carulli L, Ferrari S, Bertolini M, Tagliafico E, Rio G. 1999 Regulation of ob gene expression: Evidence for epinephrine-induced suppression in human obesity. J Clin Endocrinol Metab. 84: 3309-3312.

26. Boden G, Chen X, Kolaczynski JW, Polansky M. 1997 Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest 100:1107-1113.

27. Bramlett SB, Zhou J, Harris RBS, Handry SL, Witt TL, Zachwieja JJ. 1999 Does beta3-adrenoreceptor blockade attenuate acute exercise-induced reductions in leptin mRNA? J Appl Physiol 87:1678-1683.

28. Friedman JE, Ferrara CM, Aulak KS, Hatzoglou M, McCune SA. Park S, Sherman WM. 1997 Exercise training down-regulate ob gene expression in the genetically obese SHHF/Mcc-fa Rat. Horm Metab Res 29:214-219.

29. Pagano C, Marzolo M, Granzotto M, Ricquier D, Federspil G, Vettor R. 1999 Acute effects of exercise on circulating leptin in lean and genetically obese fa/fa rats. Biochem Biophysical Res Commu 255, 698-702.

30. Zheng D, Wooter MH, Zhou Q, Dohm GL. 1996 The effect of exercise on ob gene expression. Biochem Biophysical Res Commun 225:747-750.

31. Commins S, Marsh D, Thomas SA, Watson PM, Padgett MA, Palmiter R, Gettys TW. 1999 Norepinephrine is required for leptin effects on gene expression in brown and white adipose tissue. Endocrinology. 140: 4772-4778.

32. Pinkney JH, Coppack SW, Mohamed-Ali V. 1998 Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol 48:407-411.

33. Fogteloo AJ, Meinders AE, PIJL H, Kroon AA, Frolich M, De Leeuw PW. 2001 Renal clearance of endogenous leptin in hypertensive human with or without renal artery stenosis. Am J Physiol Endocrinol Metab. 281: E400-E404.

34. McAllister RM. 1998 Adaptations in control of blood flow with training: splanchnic and renal blood flows Med Sci Sport Exerc 30:375-381

35. Halle M, Berg A, Garwers U, Grathwohl D, Knisel W. Keul J. 1999 Concurrent reductions of serum leptin and lipids during weight loss in obese men with type II diabetes. Am J Physiol. 277:E277-E282.

36. Kohrt WM, Landt M, Birge SJ. 1996 Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older females. J Clin Endocrinol Metab 81:3980-3985.

37. Okazaki T, Himeno E, Nanri H. Ogata H, Ikeda M. 1999 Effects of mild aerobic exercise and a mild hypocaloric diet on plasma leptin in sedentary women. Clin Exp Pharm Physiol 26:415-420.

38. Pasman WJ, Sesterterp-Plantenga MS, Saris WHM. 1998 The effect of exercise training on leptin levels in obese males. Am J Physiol. 274:E280-E286.

39. Kreamer RR, Acevedo AO, Synovitz LB, Hebert EP, Gimpel T, Castracane VD. 2001 Leptin and steroid hormone responses to exercise in adolescent females runners over a 7-week season. Eur J Appl Physiol 86:85-91.

40. Hickey MS, Houmard JA, Considine RV, Tyndall GL, Midgette JB, Gavigan KE, Weidner ML, McCammon MR, Israel RG, Caro JF 1997 Gender-dependent effects of exercise training on serum leptin levels in humans. Am J Physiol Endocrinol Metab. 272:E562-566.

41. Ishii T, Yamakita T, Yamagami K, Yamamoto T, Miyamoto M, Kawasaki K, Hosoi M, Yoshioka K, Sata T, Tanaka S, Fujii S. 2001 Effect of exercise training on serum leptin levels in type 2 diabetic patients. Metabolism 50:1136-1140.

42. Cochran B Jr, Marbach EP, Poucher R, Steinberg T, Gwnup G. 1966 Effect of acute muscular exercise on serum immunoreactive insulin concentration. Diabetes. 15(11):838-41, 1966 Nov.

43. Devlin JT, Hirshman M, Horton ED, Horton ES. 1987 Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes 36:434-39.

44. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, Rothman DL, Shulman GI. 1996 Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. New Eng J Med 335:1357-1362.

45. Rogers MA, Yamamoto C, King DS, Hamberg JM, Ehsani AA, Holloszy JO. 1988 Improvement in glucose tolerance after 1 week of exercise in patients with mild NIDDM. Diabetes Care 11:613-618.

46. Bjorbaek Ch, Uotani S, Dilva B, Flier JS. 1997 Divergent signalling capacities of the long and short isoforms of the leptin receptor. J Biol Chem. 272:32686-32695.

47. Fei H, Okano HJ, Li C. et al. 1997 Anatomic localization of alternatively spliced leptin receptor (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci USA. 94:7001-7005.

48. Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415:339-343.

49. Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L. 1997 Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100:3105

50. Burks DJ, de Mora JF, Schubert M, Withers DJ, Myers MG, Towery HH, Altamuro SL, Flint CL, White MF. 2000 IRS-2 pathways intergrate female reproduction and energy homeostasis. Nature. 407:377-382.

51. Kim YB, Uotani S, Pierroz DD, Flier JS, Kahn BB. 2000 In Vivo administration of leptin activates signal transduction directly in insulin-sensitive tissues: overlapping but distinct pathways from insulin. Endocrinology 141:2328-2339.

52. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myer Jr. MG, Schwartz MW. 2001 Key enzyme in leptin-induced anorexia. Nature 413:794-795.

53. Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, Haring HU. 1997 Leptin activates PI3-Kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. Diabetologia 40:1358-1362.

54. Chibalin AV, Yu M, Ryder JW, Song XM, Galuska D, Krook A, Wallberg-Henriksson H, Zierath JR 2000 Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: Differential effects on insulin-receptor substrates 1 and 2. Proc Natl Acad Sci USA 97:38-43.

55. Musi N, Hayashi T, Fujii N, Hirshman MF, Witters LA, Goodyear LJ. 2001 AMP-activated protein kinase activity and glucose uptake in rat skeletal muscle. Am J Physiol Endocrinol Metab 280:E677-E684.

56. Kirwan JP, del Aguila LF, Hernandez JM, Williamson DL, O'Gorman DJ, Lewis R, Krishnan RK. 2000 Regular exercise enhances insulin activation of IRS-1-associated PI3-kinase in human skeletal muscle. J Appl Physiol 88:797-803.

57. Lee JS, Bruce CR, Spurrel BE, Hawley JA. 2002 Effect of training on activation of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase pathways in rat soleus muscle Clin Exp Pharmacol Physiol. 29:655-660.

58. Stephens TJ, Chen ZP, Canny BJ, Michell BJ, Kemp BE, McConell GK. 2002 Progressive increase in human skeletal muscle AMPKα2 activity and ACC phosphorylation during exercise. Am J Physiol Endocrinol Metab 282:E688-E694.

59. Steinberg GR, Rush JW, Dyck DJ 2003 AMPK expression and phosphorylation are increased in rodent muscle after chronic leptin treatment. Am J Physiol Endocrinol Metab 284:E648-654

60. Unger RH. 2002. Lipotoxic disease. Annu. Rev. Med. 53:319-336.

CHAPTER THREE (Study one)

INTACT SYMPATHETIC NERVOUS SYSTEM IS REQUIRED FOR LEPTIN EFFECTS ON RESTING METABOLIC RATE IN PEOPLE WITH SPINAL CORD INJURY

Manuscript published in Journal of Endocrinology and Metabolism 2003 88(1): 402-407

0013-7227/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(1):402–407
Copyright © 2003 by The Endocrine Society
doi: 10.1210/jc.2002-020939

Intact Sympathetic Nervous System Is Required for Leptin Effects on Resting Metabolic Rate in People with Spinal Cord Injury

JUSTIN Y. JEON, ROBERT D. STEADWARD, GARRY D. WHEELER, GORDON BELL, LINDA McCARGAR, AND VICKI HARBER

The Steadward Center, Department of Physical Education and Recreation, and Faculty of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2H9

Compared with able-bodied (AB), people with spinal cord injury (SCI) have a 3- to 5-fold higher risk of developing type 2 diabetes mellitus, which may be associated with increased fat mass. Evidence suggests that leptin regulates body adiposity through the sympathetic nervous system, which is impaired in people with high lesion SCI. The purpose of this study was to determine the relationship among leptin levels, body composition, and resting metabolic rate (RMR) in people with high lesion SCI and body mass index-, weight-, height-, and waist circumference-matched AB subjects. Fourteen subjects (seven SCI and seven AB) participated in the study. After an overnight fast, various hormones, glucose, and RMR were measured. There was no significant difference in plasma glucose, insulin, GH, cortisol, and glucagon levels between the two groups. The SCI group had 105% higher plasma leptin levels than the AB group ($P < 0.05$). Plasma leptin levels correlated with body mass index (SCI: $r = 0.80$; $P = 0.028$; AB: $r = 0.79$; $P = 0.035$) and fat mass (SCI: $r = 0.95$; $P = 0.001$; AB: $r = 78$; $P = 0.038$) in both groups. The plasma leptin level correlated with the absolute RMR (SCI: $r = 0.15$; $P = 0.75$; AB: $r = 0.99$; $P < 0.006$) and the RMR per unit fat-free mass (SCI: $r = -0.70$; $P < 0.08$; AB: $r = 0.845$; $P < 0.017$) in the AB group, but not in the SCI group. The absolute RMR was significantly reduced in the SCI group compared with the AB group, but there was no difference in the relative RMR between the groups. In conclusion, the SCI group has a significantly higher plasma leptin level than the AB group. The absolute and relative RMR correlated with leptin only in the AB group. (*J Clin Endocrinol Metab* 88: 402–407, 2003)

INDIVIDUALS WITH SPINAL cord injury (SCI) undergo abrupt changes in body composition as a result of the injury (1–3). These changes include a reduction in fat-free mass (FFM) and bone mineral density and an increase in fat mass. Consequently, people with SCI have a greater risk of developing obesity-related disorders such as cardiovascular disease and type 2 diabetes mellitus (4, 5).

Leptin, the product of the obese gene, is a 16-kDa protein primarily produced by adipocytes (6). Leptin may regulate body adiposity via central nervous system pathways that modulate both food intake and energy expenditure (7–9). Although a wide variety of central and peripheral tissues express leptin receptors (10, 11), intracerebroventricular (icv) administration of leptin is more potent than iv administration, suggesting that its target of action lies within the central nervous system (8, 12, 13). In particular, the ventromedial hypothalamus (VMH) and specific nuclei of the hypothalamus are known targets for leptin action (12, 13). In VMH-lesioned animals, food intake is increased, whereas energy expenditure is decreased, accompanied by a reduction in sympathetic nerve activity (14, 15). Satoh *et al.* (16) demonstrated that a single direct injection of leptin into the VMH caused a significant increase in plasma epinephrine and norepinephrine concentrations. Similar results were reported by Tang-Christensen *et al.* (8); circulating norepinephrine was increased by 55 ± 16% 1 h after icv leptin administration. The finding that leptin led to a significant elevation of plasma catecholamine levels provides strong evidence that central leptin administration activates the sympathetic nervous system (SNS).

Moreover, it has been reported that iv or icv administration of leptin significantly increases glucose uptake by certain tissues in mice in the absence of insulin changes (12, 13). These findings suggest that leptin-mediated glucose uptake is not due to an increase in insulin secretion. More likely, leptin-mediated glucose uptake pathways are activated by sympathetic nerves innervating the tissues. An enhanced rate of glucose uptake by brown adipose tissue in response to leptin injection into the VMH was abolished by surgical sympathetic denervation of the tissue (12). In addition, leptin suppresses glucose-induced insulin secretion via SNS activation (17). Therefore, it has been suggested that a major target site for leptin is the brain and requires an intact SNS for normal leptin action (11–13, 16–18).

People with SCI suffer decentralization of the SNS after the injury (19). Complete SCI results in a loss of motor and sensory functions via afferent and efferent spinal pathways and also in an interruption of pathways from the brain to the peripheral SNS (20–22). This interruption leads to pathological changes in sympathetic innervation through the anatomic reorganization of pathways in the spinal cord (23). As a result, leptin's influence on the regulation of energy intake and energy expenditure in people with high lesion SCI may be impaired and may increase the risk of obesity. However,

Abbreviations: AB, Able-bodied; BMI, body mass index; CV, coefficient of variation; FFM, fat-free mass; icv, intracerebroventricular; RMR, resting metabolic rate; SCI, spinal cord injury; SNS, sympathetic nervous system; VMH, ventromedial hypothalamus; WC, waist circumference.

the relationship between plasma leptin levels and resting metabolic rate (RMR) has not been investigated in people with high lesion SCI.

Thus, the purpose of this study was to identify basal leptin levels and determine the relationship among leptin levels, body composition, and RMR in an SCI group and an able-bodied (AB) group matched for age, weight, height, body mass index (BMI), and waist circumference (WC).

Subjects and Methods

Subjects

Healthy male subjects [n = 7 with complete SCI (C5–C7), SCI group; n = 7 in the AB group) agreed to participate in the study. AB subjects were matched to SCI subjects for age, weight, height, BMI, and WC. Participants were free of type 2 diabetes mellitus or coronary heart disease. An institutional ethics review board at University of Alberta approved this study. All subjects gave written informed consent to participate in the study. Subjects were asked to abstain from any strenuous physical activity during the period of study. Subject characteristics are summarized in Table 1.

Protocol

At 1800 on d 1, subjects consumed a standard meal (55% carbohydrate, 30% fat, and 15% protein) containing 12 kcal/kg body weight for lean subjects and 12 kcal/kg adjusted body weight for obese subjects (BMI, >30; adjusted body weight = ideal body weight + (actual body weight − ideal body weight) × (0.25)] (24). At 2100 on d 1, all subjects ingested a snack (140 kcal, 27 g carbohydrate, 2.7 g fat, and 1.6 g protein). At 900 on d 2, after a 12-h fast, blood samples (18 ml) were collected in heparinized tubes. Blood samples were immediately centrifuged (at 4 C) and separated, and the plasma was frozen (−80 C) until analysis was performed.

RMR

RMR was measured at 0800 h on d 2 after an 11.5-h fast. During testing all subjects were instructed to lie still and awake. Subjects rested for 30 min in a quiet room in the supine position. An adult-size canopy hood was used to collect expired air for an additional 30 min to measure RMR. The Weir equation was used to calculate RMR (25) (CPX-D, MedGraphics, Minneapolis, MN).

Body composition

Fat mass, FFM, abdominal obesity, and percent body fat were determined by a trained technician using dual energy x-ray absorptiometry (QDR 4500A, Hologic, Inc., Waltham, MA) on all subjects according to a previously published procedure (26). With the participant in the supine position, a series of transverse scans was made from head to toe at a standardized transverse scan speed of 5 cm/sec. Dual energy x-ray absorptiometry has been shown to be the most practical and accurate way to measure body composition in people with SCI (27).

Analytical procedures

All plasma samples from each subject were analyzed in duplicate in a single assay to eliminate between-assay variation. Plasma leptin (nanograms per deciliter) and glucagon (picograms per milliliter) levels were measured by RIA (human leptin RIA, Linco Research, Inc., St. Charles, MO). The intraassay coefficients of variation (CVs) were 4.7% and 7.5%, respectively. Plasma insulin (microunits per milliliter) and GH (nanograms per milliliter) levels were measured by RIA (Diagnostic Products, Los Angeles, CA). The intraassay CVs were 6.9% and 10.5%, respectively. Glucose levels were measured by the enzymatic method (Glucose Analyzer II, Beckman, Irvine, CA). Plasma epinephrine (picomoles per liter) and norepinephrine (nanomoles per liter) levels were measured by HPLC with electrochemical detection (electrochemical detector model 1045, Hewlett-Packard Co., Waldbronn, Germany) (28). The intraassay CV for these assays was 3% for epinephrine and 2.8% for norepinephrine.

Statistical analysis

Variables between the two groups were analyzed using independent *t* tests. Pearson's product-moment correlation was used to measure the strength of association between the variables. Stepwise multiple linear regression was used to determine the strongest predictor for RMR (dependent variable) from leptin, FFM, and GH (independent variables). All data were expressed as the mean ± SE. Statistical significance was set at $P < 0.05$.

Results

Body composition

Subject characteristics are listed in Table 1. Although subjects were matched for age, weight, height, BMI, and WC, the SCI group showed a significantly higher percent body fat (34.6 ± 7% *vs.* 24.4 ± 6.5%; $P = 0.016$) and lower FFM (52.7 ± 4.1 *vs.* 63.4 ± 1.1; $P = 0.03$) than the AB group.

Leptin

The 12-h fasting leptin level in the SCI group was significantly higher than that in the AB group (15.1 ± 3.8 *vs.* 7.3 ± 2.0 ng/dl; $P = 0.004$) when BMI was controlled for. When the plasma leptin levels were expressed relative to fat mass (kilograms), the SCI group still had higher plasma leptin levels than the AB group (0.45 ± 0.14 *vs.* 0.31 ± 0.15 ng/dl·kg fat mass; $P = 0.92$). The difference, however, was not statistically significant. In both groups leptin correlated with weight, WC, BMI, and fat mass (Fig. 1 and Table 2).

Catecholamine, insulin, glucose, glucagon, and GH

The SCI group had significantly lower 12-h fasting plasma epinephrine (16.3 ± 16.3 *vs.* 121.4 ± 23.3 pmol/liter; $P = 0.003$) and norepinephrine (0.6 ± 0.1 *vs.* 2.5 ± 0.3 nmol/liter) concentrations compared with the AB group ($P < 0.001$). There was no significant difference in the levels of 12-h fasting plasma insulin, glucose, glucagon, and GH between the two groups. Table 3 summarizes the hormone levels in both groups.

Leptin and RMR (Fig. 2)

Absolute RMR was lower in the SCI group compared with the AB group (SCI, 1451 ± 241; AB, 1848 ± 258 kcal/d; $P = 0.01$). However, when RMR was expressed relative to FFM, the difference was not detected between the groups (SCI, 28.3 ± 6.3; AB, 29.1 ± 3.8 kcal/d·kg FFM; $P = 0.77$; Table 4).

TABLE 1. Subject characteristics

	SCI (n = 7)	AB (n = 7)
Age (yr)	38.3 ± 3.1	38 ± 4.4
Height (cm)	178.00 ± 8.6	176.4 ± 3.1
Weight (kg)	86.5 ± 7	91.7 ± 4.3
BMI (kg/m^2)	26.7 ± 1.5	29.4 ± 1.6
Surface area (m^2)	3.2 ± 0.3	3.1 ± 0.1
Body fat (%)	34.6 ± 2.6	24.4 ± 2.5[a]
Fat mass (kg)	34.4 ± 4.5	22 ± 2.8
FFM (kg)	52.7 ± 4.1	63.4 ± 1.1[a]

Data are the mean ± SE. Body composition was measured by DEXA.
[a] $P < 0.05$.

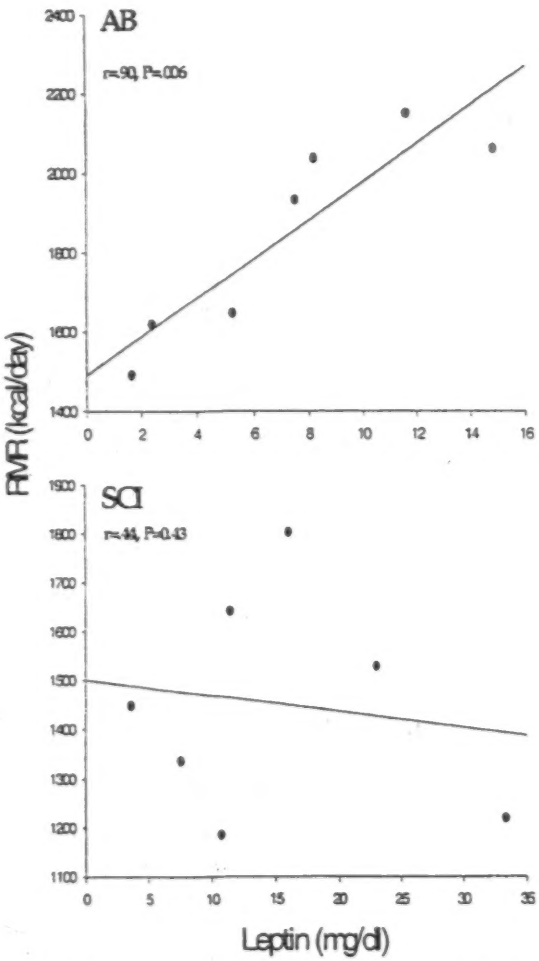


FIG. 1. Relationship between plasma leptin and fat mass in the SCI and AB groups. Leptin correlated with fat mass both in SCI ($r = 0.95$; $P < 0.001$) and AB ($r = 0.78$; $P < 0.038$) groups.

Positive correlations were found between fasting leptin levels and absolute RMR (kilocalories per day; Fig. 2) and between leptin levels and relative RMR (kilocalories per kilogram of FFM per day) in the AB group, but not in the SCI group.

Discussion

In the following study we hypothesized that 1) people with high lesion SCI would have higher plasma leptin levels compared with AB controls, and 2) plasma leptin levels would not correlate with absolute RMR and relative RMR in people with high lesion SCI. Results from the current study demonstrated that people with high lesion SCI had significantly higher plasma leptin levels compared with the AB group.

TABLE 2. Association (R) between 12-h leptin and body composition and hormones

	SCI (n = 7)	AB (n = 7)
Weight	0.93[a]	0.77[a]
WC	0.88[a]	0.78[a]
BMI	0.81[a]	0.79[a]
% Fat	0.7[b]	0.79[a]
Fat mass	0.95[a]	0.78[a]
FFM	0.86[a]	0.35
Abdominal fat	0.71[b]	0.81[a]
Insulin	0.22	0.74[b]
RMR	−0.15	0.90[a]
RMR/FFM	−0.70[b]	0.85[a]

[a] $P < 0.05$; [b] $P < 0.1$.

TABLE 3. Metabolic parameters

	SCI (n = 7)	AB (n = 7)
Glucose (mg/dl)	87.6 ± 4.2	92.3 ± 2.6
Insulin (μU/ml)	13.3 ± 3.8	13.66 ± 2.7
Leptin (ng/dl)	15.1 ± 3.8	7.3 ± 1.8[b]
GH (ng/ml)	0.4 ± 0.3	0.9 ± 0.4
Glucagon (pg/ml)	70.3 ± 9.8	77.9 ± 5.9
Epinephrine (pmol/liter)	16.3 ± 16.3	121.4 ± 23.3[a]
Norepinephrine (nmol/liter)	0.6 ± 0.1	2.5 ± 0.3[a]

Data are the mean ± SE.
[a] $P < 0.05$, [b] $P < 0.1$.

These findings are consistent with other studies (29, 30). Bauman *et al.* (29) and Huang *et al.* (30) reported that people with SCI have significantly higher plasma leptin levels than the AB controls. Higher leptin levels in people with SCI may be partly explained by a higher fat mass in the SCI group than in the AB group. A positive linear relation was found between plasma leptin levels and BMI, fat mass, percent body fat, weight, and WC in both the SCI and AB groups. Adiposity appears to predict plasma leptin levels in people with high lesion SCI as well as in the general population. In our study we found increased percent body fat in the SCI group compared with the AB group. When fat mass was controlled (plasma leptin per kilogram of fat mass), leptin levels remained 45% higher in the SCI group compared with the AB group. Although this difference was not statistically significant, these findings suggest that factors other than fat mass may also be responsible for the higher leptin levels in this group.

Recent evidence suggests that adrenergic activation may modulate leptin levels. α-Methyla-*p*-tyrosine or its more soluble methyl ester depletes tissue norepinephrine by inhibiting tyrosine hydroxylase, the initial and rate-limiting step in neuronal catecholamine synthesis (31). Intraperitoneal administration of soluble methyl ester of α-methyla-*p*-tyrosine (250 mg/kg) increased both plasma leptin (15-fold) and leptin mRNA levels in interscapular brown adipose tissue and epididymal fat (31). Additional evidence by Pinkney *et al.* (32) demonstrated that infusion of a β-adrenergic agonist (isoprenaline) rapidly reduces circulating levels of leptin. As well, Commins *et al.* (33) illustrated that leptin's effect on gene expression in brown and white adipose tissue is dependent upon norepinephrine synthesis and secretion. These results suggest the presence of a tonic inhibitory adrenergic influence on leptin secretion (31–35). Removal of this inhi-

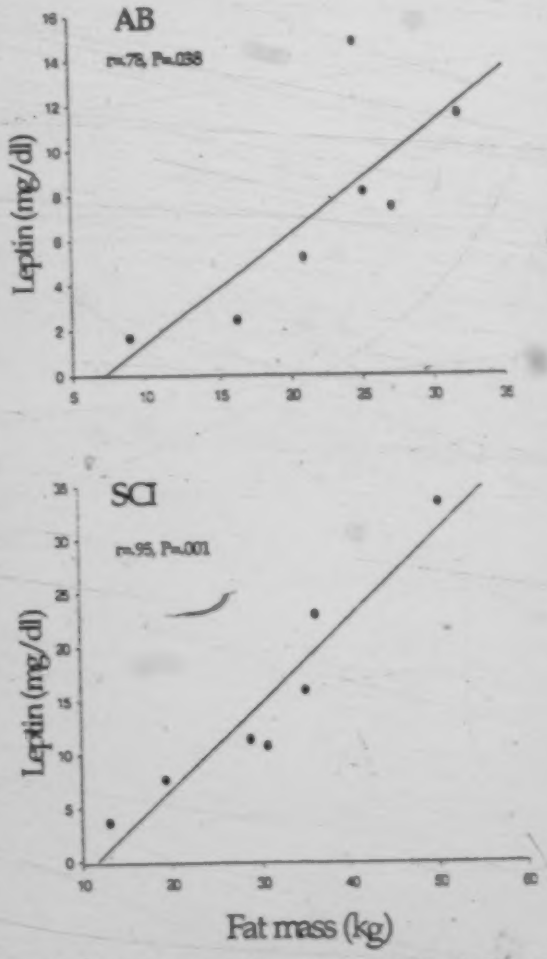


FIG. 2. Relationship between plasma leptin and RMR in the SCI and AB groupd. Leptin significantly correlated with RMR in the AB group ($r = 0.90$, $P < 0.006$), but not in SCI subjects ($r = 0.44$; $P = 0.43$).

TABLE 4. RMR

	SCI (n = 7)	AB (n = 7)
RMR (kcal/d)	1451 ± 224	1947.7 ± 26*
RMR (kcal/d · kg)	28.3 ± 6.3	29.1 ± 3.8

Data are the mean ± SE.
* $P < 0.01$.

bition would probably contribute to increased plasma leptin levels. Our data support reduced SNA accompanied by significantly lower norepinephrine levels in SCI subjects. Therefore, in addition to a higher fat mass, an impaired SNS may explain the elevated leptin levels in our SCI group.

The current study demonstrated that the SCI group had a significantly lower absolute RMR (kilocalories per day) compared with the AB group; however, when expressed per kilogram of FFM, the differences in the RMR between groups disappeared. Monroe *et al.* (36) reported significantly lower 24-h energy expenditures, RMR, and sleeping metabolic rates in people with SCI compared with the AB controls. They reported significantly lower RMR even after FFM, fat mass, and age were controlled, suggesting that reductions in peripheral SNS activity in those individuals may lead to a reduction in the overall metabolic rate. In the current study the SCI group had a significantly lower catecholamine level compared with the AB group. The lower sympathetic nerve activity in the SCI group may be related to a reduced RMR (20, 36). It has been well documented that lower sympathetic nerve activity is associated with decreased RMR in humans (37, 38). Reduced absolute RMR in the SCI group in the current study can be explained by reduced FFM and sympathetic nerve activity.

The present study showed that plasma leptin positively correlated with absolute and relative RMR in the AB group. When a multiple stepwise forward regression analysis was performed with RMR as the dependent variable and plasma leptin, FFM and GH as the independent variables, plasma leptin was the strongest predictor of RMR, accounting for 80.9% of the RMR variation. These data strongly support previous findings (39–42), which have demonstrated that a leptin level is a positive determinant of RMR in men (39), Pima children (40), women with anorexia nervosa (41), and patients with heart failure (42).

In contrast, leptin does not correlate with absolute RMR or with relative RMR in the SCI group. This result supports our hypothesis that normal SNS function is required for leptin's influence on energy expenditure. Administration of exogenous leptin or activation of the gene that encodes for leptin in normal mice (44), rats (12, 45), and rhesus monkeys (8) has resulted in increased energy expenditure, increased glucose uptake, and decreased insulin secretion. The enhanced rate of glucose uptake and the decreased insulin secretion due to administration of leptin were effectively suppressed with surgical sympathetic denervation of the tissue (12, 17). In addition, increased glucose uptake in skeletal and heart muscles after leptin injection in rats was completely prevented by pretreatment with guanethidine and not adrenal demedulation (13). Because guanethidine does not inhibit epinephrine secretion from adrenal medulla and does not affect brain norepinephrine, this result suggested that increased glucose uptake in peripheral tissue was via the SNS. Also, Monroe *et al.* (46) examined the effects of iv propranolol infusion (a β-adrenergic blocker) on RMR and demonstrated that β-adrenergic blockade decreased RMR. This suggests the presence of a tonic SNS β-adrenergic support in healthy adult humans. They suggested that the activity of the SNS was a determinant of energy expenditure, and that individuals with low resting SNS may be at risk for body weight gain because of the lower metabolic rate. It is, therefore, likely that leptin increases overall sympathetic nerve activity, leading to a significant increase in energy expenditure. Therefore, decentralization and impairment of the SNS may interrupt the pathway of leptin-mediated energy expenditure change.

Loss of association between plasma leptin levels and RMR in the SCI group, therefore, may be due to the dysfunction of SNS in this group.

Consequently, impaired or reduced activity of the SNS may put people with high lesion SCI at a higher risk of developing obesity (1–3). Peterson *et al.* (47) reported a negative correlation between body fat and plasma norepinephrine levels and suggested that decreased sympathetic activity may be a cause of obesity. Overfeeding and underfeeding in lean subjects result in significant changes in plasma norepinephrine fluxes, correlating with changes in energy expenditure (48). In addition, SNS β-adrenergic support of RMR in the healthy population accounts for approximately 71 kcal/d. In other words, those with a reduced sympathetic tone would need to compensate 26,000 kcal/yr through decreased energy intake or increased energy expenditure by non-SNS mechanisms to prevent weight gain (46).

In conclusion, the results from the present study support the hypothesis that people with high lesion SCI have higher plasma leptin levels than AB controls, and these differences are associated with increased fat mass and SNS dysfunction. To our knowledge, this is the first study to examine SNS activity, leptin levels, and RMR in people with SCI. Subjects with high lesion SCI have reduced SNS activity, which may lead to a higher risk of developing obesity and obesity-related metabolic disorders.

Acknowledgments

Received July 14, 2002. Accepted September 24, 2002.

Address all correspondence and requests for reprints to: Dr. Justin Y. Jeon, The Steadward Centre for Personal and Physical Achievement, W1-67 Van Vliet Complex, University of Alberta, Edmonton, Alberta, Canada T6G 2H9. E-mail: justin.jeon@ualberta.ca.

This work was supported by the Canadian Paraplegic Foundation, the Alberta Paraplegic Foundation (Edmonton, Canada) and the Rick Hansen Institution (Vancouver, Canada).

References

1. **Wilmet E, Ismail AA, Heilporn A, Welraeds D, Bergmann P** 1995 Longitudinal study of the bone mineral content and of soft tissue composition after spinal cord section. Paraplegia 33:674–677
2. **Jones LM, Goulding A, Gerrard DF** 1998 DEXA: a practical and accurate tool to demonstrate total and regional bone loss, lean tissue and fat mass gain in paraplegia. Spinal Cord 36:637–640
3. **Karlsson AK, Elam M, Friberg P, Sorensen PB, Sullivan L, Lonnroth P** 1997 Regulation of lipolysis by the sympathetic nervous system: a microdialysis study in normal and spinal cord injured subjects. Metabolism 46:388–394
4. **Bauman WA, Spungen AM** 1994 Disorder of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: a model of premature aging. Metabolism 43:749–756
5. **Duckworth WC, Solomon SS, Jallepall P** 1983 1983 Glucose intolerance in spinal cord injury. Arch Phys Med Rehab 64:107–110
6. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positioning cloning of the mouse obese gene and its human homologue. Nature 372:425–432
7. **Martin L, Jones P, Considine R, Su W, Boyd NF, Caro JF** 1998 Serum leptin levels and energy expenditure in normal weight women. Can J Physiol Pharmacol 76:237–241
8. **Tang-Christiansen M, Havel PJ, Jacobs B** 1999 Central administration of leptin inhibit food intake and activates the sympathetic nervous system in rhesus macaques. J Clin Endocrinol Metab 84:711–717
9. **Scarpace PJ, Matheny M, Pollock BH, Tumer N** 1997 Leptin increase uncoupling protein expression and energy expenditure. Am J Physiol 273:E226–E230
10. **Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM** 1996 Abnormal splicing of the leptin receptor in diabetic mice Nature 379:632–635
11. **Fei H, Okano HJ, Li C, Lee GH, Zhao C, Darnell R, Friedman JM** 1997 Anatomic localisation of alternatively spliced leptin receptor (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci USA 94:7001–7005
12. **Minokoshi Y, Haque MS, Shimazu T** 1999 Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes 48:287–291
13. **Haque MS, Minokoshi Y, Hamai M, Iwai H, Horiuchi M, Shimazu T** 1999 Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. Diabetes 48:176–1712
14. **Bray GA, Inoue S, Nishizawa Y** 1981 Hypothalamic obesity: the autonomic hypothesis and the lateral hypothalamus. Diabetologia 20:366–377
15. **Satoh N, Ogawa Y, Katsuura G, Tsuji T, Masuzaki H, Hiraoka J, Okazaki T, Tamaki M, Hayase M, Yoshimasa Y, Nishi S, Hosoda K, Nakao K** 1997 Pathophysiologic significance of the obese gene product, leptin, in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. Endocrinology 138:947–954
16. **Satoh N, Ebihara K, Ogawa Y, Masuzaki H, Katsuura G** 1999 Sympathetic activation of leptin via the ventromedial hypothalamus-leptin induced increase in catecholamine secretion. Diabetes 48:1787–1793
17. **Mizuno A, Murakami T, Otani S, Muwajima M, Shima K** 1998 Leptin affects pancreatic endocrine function through the sympathetic nervous system. Endocrinology 139:3863–3870
18. **Dunbar JC, Hu Y, Lu H** 1997 Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. Diabetes 46:2040–2043
19. **Karlsson AK, Friberg P, Lonnroth P, Sullivan L, Elam M** 1998 Regional sympathetic function in high spinal cord injury during mental stress and autonomic dysreflexia. Brain 121:1711–1719
20. **Schmid A, Huonker M, Barturen JM, Stahl F, Schmidt-Trucksass A** 1998 Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. J Appl Physiol 85:635–641
21. **Munakata M, Kameyama J, Kanazawa M, Nunokawa T, Moriel N, Yoshinaga K** 1997 Circadian blood pressure rhythm in patients with higher and lower spinal cord injury: simultaneous evaluation of autonomic nervous activity and physical activity. J Hypertens 15:1745–1749
22. **Schmid A, Huonker M, Stahl JM, Barturen D, Konig D** 1998 Free plasma catecholamines in spinal cord injured persons with different injury level at rest and during exercise. J Auton Nerv Syst 68:96–100
23. **Krassioukov AV, Bunge RP, Pucket WR, Bygrave MA** 1999 The changes in human spinal sympathetic preganglionic neurons after spinal cord injury. Spinal Cord 37:6–13
24. **Klein S, Horowitz JF, Landt M, Goodrick SJ, Mohamed-Ali V, Coppack SW** 2000 Leptin production during early starvation in lean and obese women. Am J Physiol 278:E280–E284
25. **Weir JBV** 1949 New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109:1–9
26. **Gingras JR, Harber V, Field CJ, McCargar LJ** 2000 Metabolic assessment of female chronic dieters with either normal or low resting energy expenditures. Am J Clin Nutr 71:1413–1420
27. **Spungen AM, Bauman WA, Wang J, Pierson JJ. RN** 1995 Measurement of body fat in individuals with tetraplegia: a comparison of eight clinical methods. Paraplegia 33:422–428
28. **Koch DD, Polskin GL** 1987 Effect of sample preparation and liquid chromatography column choice on selectivity and precision of plasma catecholamine determination. J Chromatogr 386:19–24
29. **Bauman WA, Spungen AM, Zhong YG, Mobbs CV** 1996 Plasma leptin is directly related to body adiposity in subjects with spinal cord injury. Horm Metab Res 28:730–736
30. **Huang TS, Wang YH, Chen SY** 2000 The relation of serum leptin to body mass index and to serum cortisol in men with spinal cord injury. Arch Phys Med Rehab 81:1580–1586
31. **Sivitz WI, Fink BD, Morgan DA, Fox JM, Donohoue PA, Haynes WC** 1999 Sympathetic inhibition, leptin, and uncoupling protein subtype expression in normal fasting rats. Am J Physiol 277:E668–E677
32. **Pinkney JH, Coppack SW, Mohamed-Ali V** 1998 Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol (Oxf) 48:407–411
33. **Commins SP, March DJ, Thomas SA, Watson PM, Padgett MA, Palmiter R, Gettys TW** 1999 Norepinephrine is required for leptin effects on gene expression in brown and white adipose tissue. Endocrinology 140:4772–4778
34. **Correia ML, Morgan DA, Mitchell JL, Sivitz W, Mark AL, Haynes WG** 2001 Role of corticotrophin-releasing factor in effects of leptin on sympathetic nerve activity and arterial pressure. Hypertension 38:364–368
35. **Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK** 1999 Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23:775–786
36. **Monroe MB, Tataranni PA, Pratley R, Manore MM, Skinner JS, Ravussin E** 1998 Lower daily energy expenditure as measured by a respiratory chamber in subjects with spinal cord injury compared with control subjects. Am J Clin Nutr 68:1223–1227

37. Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E, Nicod P 1993 Body fat and sympathetic nerve activity in healthy subjects. Circulation 89:2634–2640
38. Spraul M, Ravussin E, Fontvieille AM, Rising R, Larson DE, Anderson EA 1993 Reduced sympathetic nervous activity: a potential mechanism predisposing to body weight gain. J Clin Invest 92:1730–1735
39. Jorgensen J, Vahl N, Dall R, Christiansen J 1998 Resting metabolic rate in healthy adults: Relation to growth hormone status and leptin levels. Metabolism 47:1134–1139
40. Polito A, Fabbri A, Ferro-Luzzi A, Cuzzolaro M, Censi L, Ciarapica D, Fabbrini E, Giannini D 2000 Basal metabolic rate in anorexia nervosa: relation to body composition and leptin concentrations. Am J Clin Nutr 71:1495–1502
41. Solbe AD, Nicolson M, Ravussin E 1997 Total energy expenditure and the level of physical activity correlated with plasma leptin concentration in five-year-old children. J Clin Invest 99:592–595
42. Toth M, Gottlieb S, Fisher M, Ryan AS, Nichlas BJ, Poehlman ET 1997 Plasma leptin concentrations and energy expenditure in heart failure patients. Metabolism 46:450–453
43. Deleted in proof.
44. Ahren B 1999 Leptin increases circulating glucose, insulin and glucagons via sympathetic neural activation in fasted mice. Int J Obes Relat Metab Disord 23:660–665
45. Yaspelkis BB, Ansari L, Ramey E, Holland GJ, Loy SF 1999 Chronic leptin administration increases insulin mediated skeletal muscle glucose uptake and transport. Metabolism 48:671–676
46. Monroe M, Seals D, Shapiro L M, Bell C, Johnson D, Jones PK 2001 Direct evidence for tonic sympathetic nervous support of resting metabolic rate in healthy adult humans. Am J Physiol 280:E740–E744
47. Peterson HR, Rothschild M, Weinsberg CR, Fell RD, McLeish KR, Pfeifer MA 1988 Body fat and the activity of the autonomic nervous system. N Engl J Med 318:1077–1083
48. O'Dea K, Esler M, Leonard P, Stockigt JR, Nestel P Noradrenaline turnover during under- and over-eating in normal weight subjects. Metabolism 31:896–899

CHAPTER FOUR (Study two)

LEPTIN RESPONSE TO SHORT-TERM FASTING IN SYMPATHECTOMIZED MEN: ROLE OF THE SNS

Manuscript published in American Journal of Physiology Endocrinology and Metabolism 2003, 284: E634-E640

Am J Physiol Endocrinol Metab 284: E634–E640, 2003.
First published November 12, 2002; 10.1152/ajpendo.00302.2002.

Leptin response to short-term fasting in sympathectomized men: role of the SNS

JUSTIN Y. JEON,[1,2] VICKI J. HARBER,[2] AND ROBERT D. STEADWARD[1]
[1]*The Steadward Center for Personal and Physical Achievement,* [2]*Department of Physical Education and Recreation, University of Alberta, Edmonton, Alberta, Canada T6G 2H9*

Submitted 11 July 2002; accepted in final form 6 October 2002

Jeon, Justin Y., Vicki J. Harber, and Robert D. Steadward. Leptin response to short-term fasting in sympathectomized men: role of the SNS. *Am J Physiol Endocrinol Metab* 284: E634–E640, 2003. First published November 12, 2002; 10.1152/ajpendo.00302.2002.—We studied plasma leptin levels in six people with high-lesion spinal cord injury [SCI; body mass index (BMI) 25.9 ± 1.5 kg/m², age 37 ± 3.0 yr] and six able-bodied (AB) controls (BMI 29.1 ± 1.9 kg/m², age 35 ± 3.5 yr) before and after 12, 24, and 36 h of fasting. The plasma leptin levels significantly decreased during 36 h fasting by 48.8 ± 4.5% (pre: 11.3 ± 2.3, post: 6.2 ± 1.5 ng/ml) and 38.6 ± 7.9% (pre: 7.6 ± 5.0, post: 4.2 ± 1.0 ng/ml) in SCI and AB, respectively. Plasma leptin started to decrease at 24 h of fasting in the SCI group, whereas plasma leptin started to decrease at 12 h of fasting in the AB group. The current study demonstrated that plasma leptin decreased with fasting in both SCI and AB groups, with the leptin decrease being delayed in the SCI group. The delayed leptin response to fasting in the SCI group may be because of increased fat mass (%body fat, SCI: 33.8 ± 3.0, AB: 24.1 ± 2.9) and sympathetic nervous system dysfunction.

spinal cord injury; tetraplegia; *ob* gene; diabetes; sympathetic nervous system; spinal cord injury

LEPTIN, THE PRODUCT OF THE OBESE GENE, is a 16-kDa protein produced by adipocytes (58) that is delivered to the brain, primarily to the hypothalamus (6, 18). Administration of exogenous leptin to the medial basal hypothalamus in normal mice (2), rats (56), and monkeys (50) effectively decreases food intake and increases energy expenditure. Also, synthesis and secretion of leptin are increased in proportion to the degree of adiposity in several models of rodent obesity (15) and human obesity (4, 9), suggesting that leptin may represent one of the defense mechanisms against the development of obesity (30).

There is increasing evidence that another important function of leptin is to regulate the neuroendocrine response to fasting (1). In lean persons, plasma leptin concentrations decline markedly within the first 24 h of fasting, which may be important in regulating substrate metabolism and energy expenditure during early starvation (5, 17, 55). Weiss et al. (55) reported up to a 66% decline in plasma leptin levels after 1 wk of energy restriction. Decreases in the fasting-induced plasma leptin levels were prevented by maintaining euglycemia via glucose infusion during 72 h of fasting (5). Sonnenberg et al. (48) reported that plasma leptin levels declined steadily and significantly during 26 h of fasting. However, when the plasma leptin levels remained constant through glucose infusion, leptin levels did not change. These studies have provided convincing evidence that leptin has a role as an afferent signal of glucose availability to the central nervous system for modulating both long-term and short-term energy imbalance (1, 17, 54). This function of leptin defends the body from excess energy expenditure in the face of limited energy intake (1, 56).

One of the possible mechanisms of the modulation of leptin secretion during fasting may involve the sympathetic nervous system (SNS; see Ref. 14). Sivitz et al. (47) reported that the administration of α-methyl-*p*-tyrosine methyl ester (AMPT-ME) increased plasma leptin 15-fold, increased epididymal fat leptin mRNA up to 2.5-fold, increased interscapular brown adipose tissue leptin mRNA, and decreased lumbar sympathetic nerve activity. Rayner et al. (41) also presented complementary findings by showing that administration of AMPT produced hyperleptinemia in mice. Because AMPT-ME inhibits catecholamine production, the increased leptin levels after administration of AMPT-ME most likely suggest that catecholamines are required for normal leptin secretion and lack of sympathetic nerve innervation would increase leptin levels. In addition, epinephrine and norepinephrine infusion acutely decreases *ob* gene expression and plasma leptin levels (8, 39). It is assumed that a regulatory pathway involving the suppression of leptin release acts through sympathetic activation of adipose adrenergic receptors (41, 43, 47). The data suggest that the SNS may play a key role in the modulation of leptin during fasting.

People with spinal cord injury (SCI) have experienced the dysfunction of the SNS, which results in an altered hormonal and metabolic response during fasting in humans (49, 56). Palmer et al. (38) investigated

Address for reprint requests and other correspondence: J. Y. Jeon, The Steadward Center for Personal and Physical Achievement, W1-67 Van Vliet Comp, Univ. of Alberta, Edmonton AB, T6G 2H9, Canada (E-mail: justin.jeon@ualberta.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

0193-1849/03 $5.00 Copyright © 2003 the American Physiological Society

the catecholamine response to insulin-induced hypoglycemia in subjects with high-lesion SCI and in able-bodied (AB) subjects. They found that catecholamine did not increase in people with high-lesion SCI, whereas it increased >200% in AB controls. This result was confirmed by another study (32) that reported a 12.8-fold increase in norepinephrine during insulin-induced hypoglycemia in AB controls while no changes were observed in the high-lesion SCI group. Although the afferent limb of the baroreceptor arc is intact, the lesion in people with high-lesion SCI disrupts the sympathetic component of the efferent limb in the cervical spinal cord. This results in their inability to increase sympathetic nerve activity during fasting (32).

Because intact SNS is required for normal leptin action (7, 8) and catecholamine response to fasting, it is important to determine the leptin response to short-term fasting in people with high-lesion SCI. However, leptin response to fasting has not been investigated in people with SCI whose SNS is impaired. Therefore, the purpose of the study was to determine the leptin response to 0, 12, 24, and 36 h of fasting in the SCI and AB groups.

METHODS

Subjects. Six male subjects with complete SCI (C_5–C_7, complete lesion) and six male AB subjects participated in the study. Subjects were matched by age, weight, height, body mass index (BMI), and waist circumference, and no participants had type 2 diabetes mellitus or coronary heart disease. Written informed consent was obtained before participation in the study, which was approved by the University of Alberta Ethics Review Board. Subjects were asked to abstain from any strenuous physical activity during the period of study. Subject characteristics are summarized in Table 1.

Experimental procedure. At 1800, subjects consumed a standard meal (55% carbohydrate, 30% fat, and 15% protein) containing 12 kcal/kg body wt for lean subjects and 12 kcal/kg adjusted body weight for obese subjects [BMI >30; adjusted body wt = ideal body wt + (actual body wt − ideal body wt × 0.25); see Ref. 31]. At 2100, a snack (140 kcal, 27 g carbohydrate, 2.7 g fat, and 8.8 g protein) was ingested by every subject. After the snack, subjects were asked not to consume any food except water until 0900 of *day 3*. At 0900 and 2100 of *day 2* and 0900 of *day 3* (12, 24, and 36 h of fasting, respectively), subjects were asked to come to the laboratory, and blood samples (18 ml) were collected in heparinized tubes. Blood samples were centrifuged immediately (4°C), separated, and frozen (−80°C) until an analysis was performed.

Table 1. *Characteristics of subjects*

	SCI (n = 6)	AB (n = 6)	P Value
Age, yr	37.0 ± 3.0	35 ± 3.5	0.47
Height, cm	175.8 ± 2.9	176.6 ± 1.4	0.80
Weight, kg	81.6 ± 6.0	90.7 ± 5.0	0.27
BMI, kg/m^2	25.9 ± 1.5	29.1 ± 1.9	0.22
Body fat, %	33.8 ± 3.0	24.1 ± 2.9	0.04
Fat mass, kg	27.1 ± 3.7	21.6 ± 3.3	0.30
FFM, kg	49.4 ± 2.3	63.2 ± 1.3	<0.01
Leptin, ng/ml	12.0 ± 2.8	6.1 ± 1.5	0.09
Glucose, mg/dl	89.0 ± 4.7	92.2 ± 3.0	0.58
Insulin, μU/ml	12.8 ± 4.4	12.5 ± 2.9	0.97
GH, ng/ml	0.4 ± 3.0	0.9 ± 0.4	0.06
Epi, pmol/l	19.0 ± 19.0	124.5 ± 27.4	0.01
NE, nmol/l	0.5 ± 0.2	2.3 ± 0.3	<0.01
RMR, kcal/day	1,489 ± 89	1,812.7 ± 107.5	0.04
RMR/FFM, kcal/day^{-1}·kg^{-1}	30.3 ± 1.6	28.7 ± 1.6	0.49

Data are means ± SE; n, no. of subjects. SCI, spinal cord injury; AB, able bodied; BMI, body mass index; FFM, fat-free mass; Epi, epinephrine; NE, norepinephrine; GH, growth hormone; RMR, resting metabolic rate.

Resting metabolic rate. Oxygen consumption at rest was used to calculate the resting metabolic rate (RMR) and fuel utilization. RMR were measured at 0800 of *day 2* and *day 3*. The subjects rested in a supine position for 30 min before measurement. A transparent canopy was placed over the heads of the subjects while oxygen consumption was measured. Subjects were not permitted to sleep or move during the testing. Energy expenditure was calculated using the Weir equation (model CPX-D; MedGraphics, Minneapolis, MN; see Ref. 54).

Body composition. Fat mass, fat-free mass (FFM), abdominal obesity, and percent body fat were determined by a trained technician using dual-energy X-ray absorptiometry (DEXA; Hologic QDR 4500A; Hologic, Waltham, MA) on all subjects according to a previously published procedure (21). With the participant lying supine, and with all metal objects removed, a series of transverse scans was made from head to toe at a standardized transverse scan speed of 5 cm/s. DEXA has been shown to be the most practical and accurate way to measure body composition in people with SCI (21).

Analytical procedures. All samples from each subject were analyzed in one assay to avoid between-assay variation. Plasma leptin levels (ng/ml) were measured in duplicate at each time point by RIA (Human leptin RIA; Linco Research, St. Charles, MO). The limit of sensitivity was 0.5 ng/ml. The intra-assay coefficient of variation (CV) was 4.7%. Plasma insulin levels (μU/ml) were measured in duplicate at each time point by RIA (Diagnostic Products, Los Angeles, CA). The intra-assay CV was 6.9%. Growth hormone (GH) levels were measured in duplicate at each time point by RIA (Diagnostic Products). The intra-assay CV was 10.5%. Glucose levels were measured by the enzymatic method (Glucose analyzer II; Beckman Instrument, Irvine, CA). Plasma epinephrine (pmol/l) and norepinephrine (nmol/l) levels were measured by HPLC with electrochemical detection (electrochemical detector model 1045; Hewlett-Packard, Waldbronn, Germany; see Ref. 26). The intra-assay CV for these assays was 7% for epinephrine and 9.8% for norepinephrine.

Statistical analysis. The statistical analysis was performed by a two-way ANOVA repeated measurement; between-groups (SCI vs. AB) and within-subject (0, 12, 24, and 36 h of fasting) factors and their interaction were considered. Comparisons among the variables between the two groups were made by independent t-tests. Pearson's product-moment correlation was used to measure the strength of association between the variables. Stepwise multiple linear regression was used to determine the strongest predictor for RMR (dependent variable) among leptin, FFM, and GH (independent variables). All data have been expressed as means ± SE. Statistical significance was set for $P < 0.05$.

RESULTS

Plasma leptin response to fasting. At 0 h of fasting, plasma leptin was higher in the SCI group compared

with the AB group; however, the difference did not reach statistical significance. During the 36-h fasting period, plasma leptin decreased significantly below the prefasting level in both the SCI (0 h fasting: 11.34 ± 2.27 vs. 36 h fasting: 6.16 ± 1.46 ng/ml) and the AB (0 h: 7.58. ± 2.05 vs. 36 h: 4.18 ± 1.01 ng/ml) groups. Plasma leptin started to decrease at 24 h and continued to decrease until 36 h of fasting in the SCI group, whereas it started to decrease at 12 h and started to plateau after 24 h of fasting in the AB group (Fig. 1). When the BMI was controlled, the two-way ANOVA repeated measurement showed an interaction between leptin responses to fasting in the groups ($P = 0.011$).

Plasma hormone and metabolite response to fasting. There was no significant difference in plasma glucose, insulin, cortisol, and GH before fasting between the groups. There was no significant difference in the hormones and the glucose response during 36 h of fasting in either group.

RMR. Absolute RMR (kcal/day) was higher in the AB vs. SCI group at both 12 and 36 h of fasting. However, there were no differences between the groups in relative RMR (kcal·day^{-1}·kg FFM^{-1}) at either 12 or 36 h of fasting. Over the 36-h period of fasting, RMR (absolute and relative) values did not change in either group.

Relationship between RMR and plasma leptin during fasting. Positive correlations were found between leptin levels and RMR (absolute and relative) in the AB group. On the other hand, no correlations were found between leptin levels and RMR (absolute and relative) in the SCI group at either 12 or 36 h of fasting (Fig. 2).

Body composition. Although subjects were matched for age, weight, height, BMI, and waist circumference, the SCI group showed a significantly higher percentage of body fat (33.8 ± 3.0 vs. 24.4 ± 2.5%), resulting from higher fat mass (27.1 ± 3.7 vs. 21.6 ± 3.3) and lower FFM (49.4 ± 2.3 vs. 63.41 ± 2.96), compared with the AB group (Table 2).

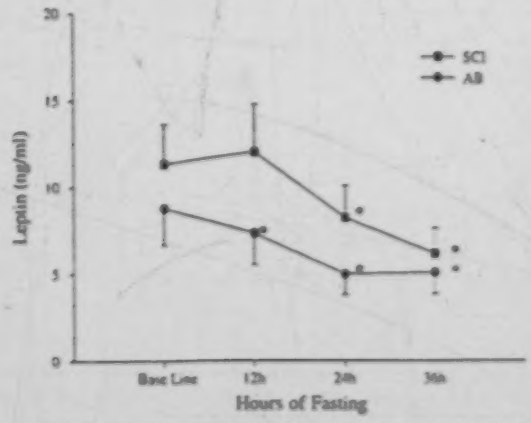


Fig. 1. Plasma leptin response to 36 h of fasting. SCI, spinal cord injury; AB, able bodied. *$P < 0.05$ compared with baseline.

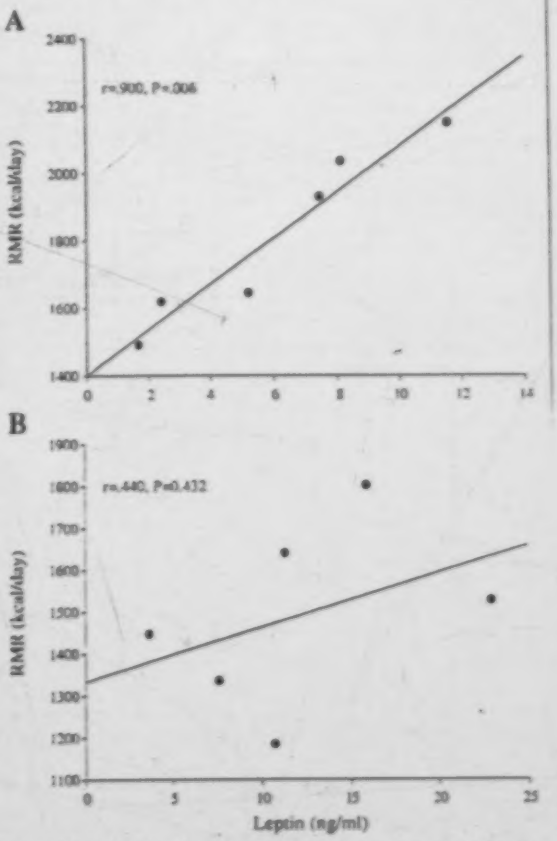


Fig. 2. Relationship between leptin and resting metabolic rate (RMR) in AB (*A*) and SCI (*B*) groups.

DISCUSSION

To our knowledge, the present study was the first to examine the leptin response to a 36-h fast in SCI and AB individuals. We demonstrated that leptin levels declined in both groups but was delayed in the SCI volunteers.

Leptin response to short-term fasting. A fasting-induced decrease in plasma leptin plays an important role in initiating changes in substrate metabolism and energy expenditure (44, 50). In humans, a significant reduction of energy intake or fasting suppresses leptin levels to a much greater extent than would be expected on the basis of changes in the adipose tissue mass (5, 17, 24). This is consistent with evidence that leptin has a role as a signal of energy deficiency and an integrator of neuroendocrine function (1). The present study demonstrated that plasma leptin decreased with 36 h of fasting by 48.8 ± 4.5 and 38.6 ± 7.9% in both the SCI and the AB groups, respectively. The literature in this area suggests that short-term fasting (12 h) reduces plasma leptin levels in the AB population (5, 29). Our

Table 2. *RMR*

	SCI Group (n = 6)		AB Group (n = 7)	
	Day 1	*Day 2*	*Day 1*	*Day 2*
RMR, kcal/day	1,489 ± 89	1,411 ± 69	1,812.17 ± 107.5*	1,896 ± 108.9*
RMR/FFM, kcal·day^{-1}·kg FFM^{-1}	30.26 ± 1.6	28.9 ± 2	28.67 ± 1.61	30 ± 1.6

Data are means ± SE; n, no. of subjects. *$P < 0.05$ between groups.

data are consistent with these findings. Plasma leptin was reduced significantly after 12 h of fasting in the AB group but was not altered until 24 h in the SCI group. The mechanism for the delayed leptin response in the SCI group may be related to SNS dysfunction and adiposity level.

Leptin mediates many of its physiological effects by activating neurons in the hypothalamus that innervate and stimulate sympathetic preganglionic neurons in the spinal cord. In individuals with high-lesion SCI, these neurons are atrophied (28), and stimulation below the lesion may be impaired. Consequently, impairment of the SNS may alter leptin secretion and action (11, 12, 18, 33, 34). Stimulation of the SNS by infusing catecholamine or isoprenaline decreases leptin production and leptin expression in peripheral tissues (7, 8, 39). In addition, sympathetic blockade or chemical sympathectomy increases circulating leptin (41). These results suggest that a tonic inhibitory adrenergic modulation influences leptin action and secretion (24, 27, 28). Hence, removal of this inhibition, as a result of SCI, may lead to an abnormal leptin response to fasting in SCI. Therefore, the delayed leptin response during fasting in the SCI group may be because of an impairment of the SNS system.

In addition to SNS dysfunction, the significantly higher percentage of body fat in our SCI group may be another factor contributing to the delayed leptin response to fasting. Excessive adiposity is known to reduce whole body lipolytic sensitivity, and obese individuals have a blunted leptin response to food restriction (19, 20, 25, 24). Fasting-induced secretion of epinephrine stimulates adipose tissue β-adrenergic receptors and leads to increased lipolysis and decreased leptin concentrations (19, 20, 24). The failure of circulating levels of epinephrine to increase in our SCI subjects is likely because of their SNS dysfunction. Furthermore, their higher levels of body fat could further reduce lipolytic sensitivity (19, 20, 25). These results suggest that the delayed leptin decline during fasting in the SCI group may be because of both SNS dysfunction and decreased lipolytic sensitivity accompanied by increased fat mass.

Mechanisms for fasting-induced leptin decline. Both the SCI and AB groups experienced a significant decrease in plasma leptin levels, although the SCI group showed a delayed leptin response to fasting, which may suggest that adrenergic mechanisms are not the sole mechanism of leptin production and/or regulation during fasting. For example, evidence suggests that glucose metabolism is partly responsible for leptin production in humans (5, 17). The importance of glucose metabolism in regulating the level of leptin was demonstrated by Boden et al. (5), who found that glucose infusion during fasting prevented the fast-induced leptin decline. Also, Sonnenberg et al. (48) reported that plasma leptin levels declined steadily and significantly during 26 h of fasting. When the plasma glucose levels remained constant between 104 and 117 mg/dl via glucose infusion, the leptin level did not change during fasting. The plasma leptin even increased during 26 h of fasting when glucose levels were increased >157 mg/dl via glucose infusion. These studies have provided convincing evidence that the plasma glucose level is one of the major contributors for leptin production. Wang et al. (53) suggested that glucoregulation of leptin production may be mediated by the entry of glucose in the glucosamine pathway via L-glutamine, the D-fructose-6-phosphate amidotransferase step.

Another leptin-regulatory factor during fasting may be circulating insulin. Barr et al. (3) showed that treatment of rat adipocytes with insulin increased tissue leptin content and secretion during a 2-h incubation period. Also, insulin administration increased leptin release within 10 min, suggesting that insulin may act as a leptin secretagogue (27). Other studies have demonstrated that leptin administration inhibited insulin secretion (35). Our findings would support the hypothesis of an SNS-mediated leptin-insulin interaction. There was a positive correlation between the 36-h fasting leptin levels and the 36-h fasting insulin levels in the AB group ($r = 0.83$, $P = 0.04$), but no similar correlation was identified in the SCI group ($r = 0.61$, $P = 0.20$).

Relationship between plasma leptin and RMR. The present study showed that plasma leptin positively correlated with RMR (absolute and relative) in the AB group. When a multiple stepwise forward regression analysis was performed with the RMR as the dependent variable and with the plasma leptin, FFM, and GH as the independent variables, plasma leptin was the strongest predictor of RMR, accounting for 74.5% of the RMR variation. Together with the previous demonstration that the leptin level is a positive determinant of RMR in men, Pima children, women with anorexia nervosa, and patients with heart failure (22, 31, 40, 42, 51), the present study supports the hypothesis that leptin is a significant factor in the regulation of energy expenditure in humans. In contrast, leptin was not correlated with RMR (absolute and relative) in the SCI group. This result supports the hypothesis that normal SNS function is required for leptin to influence energy expenditure. Administration of exogenous leptin or activation of the gene that encodes for leptin in

normal mice (2), rats (35, 56), and rhesus monkeys (50) has resulted in increased energy expenditure, increased glucose uptake, and decreased insulin secretion. The enhanced rate of glucose uptake and decreased insulin secretion resulting from administration of leptin was suppressed effectively with surgical sympathetic denervation of the tissue (18, 33–35). Also, increased glucose uptake in skeletal and heart muscles was prevented completely by pretreatment with guanethidine but not by adrenal demedullation (18). Because guanethidine does not inhibit secretion of epinephrine from the adrenal medulla and does not affect brain norepinephrine, these results suggest that the SNS mediates the effects of leptin.

Monroe et al. (36) examined the effects of intravenous propranolol infusion, a β-adrenergic blocker, on RMR. They demonstrated that β-adrenergic blockade acutely decreased RMR, suggesting that a tonic SNS β-adrenergic effect in healthy adult humans was operating. The literature also suggested that the activity of the SNS was a determinant of energy expenditure and that individuals with low resting SNS may be at risk for body weight gain because of the lower metabolic rate (45). Therefore, it is likely that leptin increases overall sympathetic nerve activity, thereby leading to a significant increase in energy expenditure. It follows that the decentralization of the SNS may interrupt the pathway of leptin-mediated energy expenditure regulation in this study. Therefore, the lack of association between leptin and RMR in the SCI group in this study may be because of the dysfunction of the SNS.

The effect of short-term fasting on RMR is controversial (57). Hypoglycemia is known to increase the level of SNS activity and increase the release of norepinephrine (38), which in turn causes an increased rate of lipolysis to compensate for the decreased level of glucose (20, 24). The increased level of norepinephrine resulting from hypoglycemia would also increase energy expenditure (36). However, it has also been demonstrated that hypoglycemia decreases circulating leptin levels in animal and human models (5, 24), which would result in decreased SNS activity. Because norepinephrine is a marker of the level of SNS activity, decreased SNS activity would theoretically decrease energy expenditure (37). Thus the response of SNS to fasting is paradoxical and requires further investigation and explanation. Our study suggests that RMR does not change with 36 h of fasting in either the AB or SCI group compared with the level obtained from 12 h of fasting in both SCI and AB groups. However, Zauner et al. (57) reported that RMR in short-term fasting is increased as a result of an increase in serum norepinephrine. They measured resting energy expenditure after 36, 60, and 84 h of fasting and found that norepinephrine concentration increased from 1,716 ± 574 to 3,728 ± 1,636 pmol/l. The resting energy expenditure was increased from 3.97 ± 0.9 kJ/min at 12 h of fasting to 4.53 ± 0.9 kJ/min at 84 h of fasting.

In conclusion, the leptin decline in response to short-term fasting is delayed in the SCI group. The altered response in leptin secretion in the SCI group with short-term fasting may be because of SNS dysfunction and increased adiposity. However, other mechanisms may influence leptin production during fasting (i.e., glucose and insulin levels). Leptin was a strong determinant of energy expenditure in the AB group, which suggests that leptin-mediated regulation of energy expenditure requires an intact SNS.

This study was funded by the Alberta Paraplegic Foundation, Edmonton, AB, Canada, and Rick Hansen Institution, Vancouver, BC, Canada.

REFERENCES

1. **Ahima RS.** Role of leptin in the neuroendocrine response to fasting. *Nature* 382: 250–252, 1996.
2. **Ahren B.** Leptin increases circulating glucose, insulin and glucagons via sympathetic neural activation in fasted mice. *Int J Obes Relat Metab Disord* 23: 660–665, 1999.
3. **Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW.** Insulin stimulates both leptin secretion and production by rat white adipose tissue. *Endocrinology* 138: 4463–4472, 1997.
4. **Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, and Rascher W.** Plasma leptin levels in healthy children and adolescents: dependence on body mass index, fat mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 82: 2904–2910, 1997.
5. **Boden G, Chen X, Mozzoli M, and Ryan I.** Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 81: 3419–3423, 1996.
6. **Bray GA, Inoue S, and Nishizawa Y.** Hypothalamic obesity: the autonomic hypothesis, and the lateral hypothalamus. *Diabetologia* 20: 366–377, 1981.
7. **Carulli L, Ferrari S, Bertolini M, Tagliafico E, and Rio G.** Regulation of ob gene expression: evidence for epinephrine-induced suppression in human obesity. *J Clin Endocrinol Metab* 84: 3309–3312, 1999.
8. **Commins S, Marsh D, Thomas S, Watson PM, Padgett M, Palmiter R, and Gettys TW.** Norepinephrine is required for leptin effects on gene expression in brown and white adipose tissue. *Endocrinology* 140: 4772–4778, 1999.
9. **Considine RV, Sinha MK, Heiman ML, Kriaugiunas A. & Stephens TW, and Nyce MR.** Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292–295, 1996.
10. **Corrall JM, Frier BM, McClemont JW, Taylor SJ, and Christie NE.** Recovery mechanisms from acute hypoglycemia in complete tetraplegia. *Paraplegia* 17: 314–318, 1980.
11. **Correia MLG, Morgan DA, Mitchell JL, Sivitz WI, Mark AL, and Haynes WG.** Role of corticotrophine-releasing factor in effects of leptin on sympathetic nerve activity and arterial pressure. *Hypertension* 38:384–388, 2001.
12. **Dunbar JC, Hu Y, and Lu H.** Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* 46: 2040–2043, 1997.
13. **Evan BA, Agar L, and Summers RJ.** The role of the sympathetic nervous system in the regulation of leptin synthesis in C57BL/6 mice. *FEBS Lett* 444: 149–154, 1999.
14. **Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, and Elmquist JK.** Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 21: 1375–1385, 1998.
15. **Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, and Flier JS.** Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1: 1311–1314, 1995.
16. **Gingras JR, Harber V, Field CJ, and McCargar LJ.** Metabolic assessment of female chronic dieters with either normal or low resting energy expenditures. *Am J Clin Nutr* 71: 1413–1420, 2000.
17. **Grinspoon S, Askari H, Landt ML, Nathan DM, Schoenfeld DA, Hayden DL, Laposata M, Hubbard J, and Klibanski A.**

Effects of fasting and glucose infusion on basal and overnight leptin concentration in normal-weight women. *Am J Clin Nutr* 66: 1352–1356, 1997.

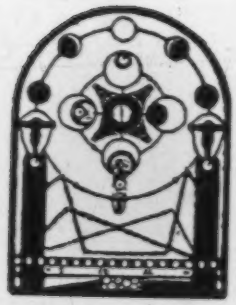
18. **Haque MS, Minokoshi Y, Hamai M, Iwai H, Horiuchi M, and Shimazu T.** Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. *Diabetes* 48: 176–1712, 1999.
19. **Horowitz JF, Coppack SW, Paramore D, Cryer PE, Zhao G, and Klein S.** Effect of short-term fasting on lipid kinetics in lean and obese women. *Am J Physiol Endocrinol Metab* 276: E278–E284, 1999.
20. **Horowitz JF and Klein S.** Whole body and abdominal lipolytic sensitivity to epinephrine is suppressed in upper body obese women. *Am J Physiol Endocrinol Metab* 278: E1144–E1152, 2000.
21. **Jones L, Goulding M, and Gerrard DF.** DEXA: a practical and accurate tool to demonstrate total and regional bone loss, lean tissue and fat mass gain in paraplegia. *Spinal Cord* 36: 637–640, 1998.
22. **Jorgensen J, Vahl N, Dall R, and Christiansen J.** Resting metabolic rate in healthy adults: relation to growth hormone status and leptin levels. *Metabolism* 47: 1134–1139, 1998.
23. **Karlsson AK, Friberg P, Lonnroth P, Sullivan L, and Elam M.** Regional sympathetic function in high spinal cord injury during mental stress and autonomic dysreflexia. *Brain* 121: 1711–1719, 1998.
24. **Klein S, Horowitz JF, Landt M, Goodrick SJ, and Mohamed-Ali V, and Coppack SW.** Leptin production during early starvation in lean and obese women. *Am J Physiol Endocrinol Metab* 278: E280–E284, 2000.
25. **Klein S, Sakurai Y, Romijn JA, and Carroll RM.** Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. *Am J Physiol Endocrinol Metab* 265: E801–E806, 1993.
26. **Koch DD and Polzin GL.** Effect of sample preparation and liquid chromatography column choice on selectivity and precision of plasma catecholamine determination. *J Chromatogr A* 386: 19–24, 1987.
27. **Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, and Caro JF.** Acute, and chronic effects of insulin on leptin production in humans: studies on vivo and in vitro. *Diabetes* 45: 699–701, 1996.
28. **Krassioukov AV, Bunge RP, Pucket WR, and Bygrave MA.** The changes in human spinal sympathetic preganglionic neurons after spinal cord injury. *Spinal Cord* 37: 6–13, 1999.
29. **Maccario M, Aimaretti G, Gorneli G, Gauna C, Grottoli S, Bidlingmaier M, Strasburger CJ, Dieguez C, Casanueva FF, and Ghigo E.** Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans. *Am J Physiol Endocrinol Metab* 279: E411–E416, 2000.
30. **Maffei MJ, Halaas J, Ravussin E, Pratley RE, Lee GM, Zhang Y, Fei H, Kim S, Lallone R, and Ranganathan S.** Leptin levels in human and rodent: measurement of plasma leptin and ob mRNA in obese and weight-reduced subjects. *Nat Med* 1: 1155–1161, 1995.
31. **Martin L, Jones P, and Considine R.** Serum leptin levels and energy expenditure in normal weight women. *Can J Physiol Pharmacol* 76: 237–241, 1998.
32. **Mathias CJ, Frankel HL, Turner RC, and Christensen NJ.** Physiological responses to insulin hypoglycemia in spinal man. *Paraplegia* 17: 319–326, 1980.
33. **Minokoshi Y, Haque MS, and Shimazu T.** Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes* 48: 287–291, 1999.
34. **Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, and Kahn BB.** Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415: 339–343, 2002.
35. **Mizuno A, Murakami T, Otani S, Muwajima M, and Shima K.** Leptin affects pancreatic endocrine function through the sympathetic nervous system. *Endocrinology* 139: 3863–3870, 1998.
36. **Monroe M, Seals D, Shapiro L, Bell C, Johnson D, and Jones PP.** Direct evidence for tonic sympathetic nervous support of resting metabolic rate in healthy adult humans. *Am J Physiol Endocrinol Metab* 280: E740–E744, 2001.
37. **Monroe MB, Tataranni PA, Pratley R, Manore MM, Skinner JS, and Ravussin E.** Lower daily energy expenditure as measured by a respiratory chamber in subjects with spinal cord injury compared with control subjects. *Am J Clin Nutr* 68: 1223–1227, 1998.
38. **Palmer JP, Henry DP, Benson JW, Johnson DG, and Ensinck JW.** Glucagon response to hypoglycemia in sympathectomized man. *J Clin Invest* 57: 522–525, 1976.
39. **Pinkney JH, Coppack SW, and Mohamed-Ali V.** Effect of isoprenaline on plasma leptin, and lipolysis in humans. *Clin Endocrinol (Oxf)* 48: 407–411, 1998.
40. **Polito A, Fabbri A, Ferro-Luzzi A, Cuzzolaro M, Censi L, Ciarapica D, Fabbrini E, and Giannini D.** Basal metabolic rate in anorexia nervosa: relation to body composition and leptin concentrations. *Am J Clin Nutr* 71: 1495–1502, 2000.
41. **Rayner DV, Simon E, Duncan JS, and Trayhurn P.** Hyperleptinaemia in mice induced by administration of the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine. *FEBS Lett* 429: 395–398, 1998.
42. **Salbe AD, Nicolson M, and Ravussin E.** Total energy expenditure and the level of physical activity correlated with plasma leptin concentration in five-year-old children. *J Clin Invest* 99: 592–595, 1997.
43. **Satoh N, Ebihara K, Ogawa Y, Masuzaki H, and Katsuura G.** Sympathetic activation of leptin via the ventromedial hypothalamus-leptin induced increase in catecholamine secretion. *Diabetes* 48: 1787–1793, 1999.
44. **Scarpace PJ, Matheny M, Pollock BH, and Tumer N.** Leptin increases uncoupling protein expression and energy expenditure. *Am J Physiol Endocrinol Metab* 273: E226–E230, 1997.
45. **Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E, and Nicod P.** Body fat and sympathetic nerve activity in healthy subjects. *Circulation* 89: 2634–2640, 1993.
46. **Schmid A, Huonker M, Stahl JM, Barturen D, and Konig D.** Free plasma catecholamines in spinal cord injured persons with different injury level at rest and during exercise. *J Auton Nerv Syst* 68: 96–100, 1998.
47. **Sivitz WI, Fink BD, Morgan DA, Fox JM, Donohoue PA, and Haynes WG.** Sympathetic inhibition, leptin, and uncoupling protein subtype expression in normal fasting rats. *Am J Physiol Endocrinol Metab* 277: E668–E677, 1999.
48. **Sonnenberg GE, Krakower GR, Hoffmann RG, Maas DL, Hennes MMI, Kissebah AH.** Plasma leptin concentration during extended fasting and graded glucose infusions: Relationships with changes in glucose, insulin and FFA. *J Clin Endocrinol Metab* 86: 4895–4900, 2001.
49. **Spungen AM, Bauman WA, Wang J, Pierson RN Jr.** Measurement of body fat in individuals with tetraplegia: a comparison of eight clinical methods. *Paraplegia* 33: 402–408, 1995.
50. **Tang-Christiansen M, Havel PJ, Jacobs R, Larsen PJ, and Cameron JL.** Central administration of leptin inhibits food intake and activates the sympathetic nervous system in *Rhesus Macaques*. *J Clin Endocrinol Metab* 84: 711–717, 1999.
51. **Toth M, Gottlieb S, Fisher M, Ryan AS, Nicklas BJ, and Poehlman ET.** Plasma leptin concentrations and energy expenditure in heart failure patients. *Metabolism* 48: 450–453, 1997.
52. **Wahrenberg H, Lonnqvist F, and Arner P.** Mechanisms underlying regional differences in lipolysis in human adipose tissue. *J Clin Invest* 84: 458–469, 1989.
53. **Wang Q, Bing C, Al-Barazanji K, Mossakowaska DE, Wang XM, McBay DL, Neville WA, Taddayon M, Pickavance L, Dryden S, Thomas ME, McHale MT, Gloyer IS, Wilson S, Buckingham R, Arch JR, Trayhurn P, and Williams G.** Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* 46: 335–341, 1997.
54. **Weir JBV.** New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 109: 1–9, 1949.

55. **Weiss BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R, and Gougeon R.** Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentration in obese women. *Am J Clin Nutr* 70: 321–330, 1999.

56. **Yaspelkis BB, Ansari L, Ramey E, Holland GJ, and Loy SF.** Chronic leptin administration increases insulin mediated skeletal muscle glucose uptake and transport. *Metabolism* 48: 671–676, 1999.

57. **Zauner C, Schneeweiss B, Kranz A, Madl C, Ratheiser K, Kramer L, Roth E, Schneider B, and Lenz K.** Resting energy expenditure in short-term starvation is increased as a result of an increase in serum norepinephrine. *Am J Clin Nutr* 71: 1511–1515, 2000.

58. **Zhang Y, Proenca R, Maffei M, Barone M, Peopold L, and Friedman JM.** Positioning cloning of the mouse obese gene, and its human homologue. *Nature* 372: 425–432, 1994.



CHAPTER FIVE

EFFECTS OF TRAINING WITH FES-ROWING ON PLASMA LEPTIN LEVELS IN PEOPLE WITH SPINAL CORD INJURY

This chapter was prepared for publication in American Journal of Physiology.

INTRODUCTION

Despite the medical problem of obesity throughout the world, one of the most interesting facts is the stability of body weight over lengthy periods of time (34, 43). Body weight is tightly regulated by a feedback loop, which maintains body weight homeostasis (43). Identification of the *ob* gene using positional cloning and the characterization of the hormone leptin has greatly increased the understanding of body weight control (1, 25, 105). Leptin is produced by adipose tissue and affects food intake, energy expenditure, and neuroendocrine status (1, 2, 30). Leptin deficiency, as seen in *ob/ob* mice and humans with congenital leptin deficiency, contributes to obesity (25, 30). Lack of leptin in circulation is perceived by the hypothalamus as an energy deficient status. In response, the hypothalamus secretes neuropeptide mediators of leptin action such as neuropeptide Y (NPY) and melanin-concentrating hormone (MCH), causing increased energy intake and decreased energy expenditure, contributing to obesity (1, 14, 22, 83). Conversely, the administration of leptin to *ob/ob* mice and children with congenital leptin deficiency decreases feeding, normalizes body fat and body temperature, and also corrects neuroendocrine abnormalities (9, 25, 30). Studies report that food intake is reduced by 30-31% and glucose uptake increased up to 27% in response to leptin administration in leptin-treated animals as compared to a control group (103). Although leptin deficiency is associated with morbid obesity in some animals and humans, most obese humans generally have elevated circulating leptin levels, which suggests that in general, human obesity is related to leptin resistance rather than leptin deficiency (16, 60).

In addition to leptin's role as an anti-obesity hormone, another important role that leptin plays is as an anti-starvation hormone (2). Plasma leptin regulates the neuroendocrine response to starvation (6, 49). In lean individuals, plasma leptin concentrations decline markedly within the first 12 hrs of fasting (6). Also, we recently demonstrated that plasma leptin levels were reduced by 48.8±4.5% and 38.6±7.9% within 36 hrs in both spinal cord injury (SCI) subjects and able-bodied (AB) subjects, respectively (39). Weiss et al. (100) reported up to a 66% decline in plasma leptin levels after one week of energy restriction, and may be important in regulating substrate metabolism and energy expenditure during early starvation (2, 39, 59). However, a

decrease in fasting-induced plasma leptin was prevented by maintaining euglycemia via glucose infusion during a 72-h fasting period (6). Sonnenberg et al. (87) reported that plasma leptin levels declined steadily and significantly during 26 hr of fasting. However, when plasma glucose levels remained constant through glucose infusion, leptin levels did not change. These studies have provided convincing evidence that leptin has a role as an afferent signal of glucose availability to the central nervous system for modulating both long-term and short term energy imbalance. This function of leptin defends the body from excess energy expenditure in the face of limited energy intake.

In contrast to the consistent plasma leptin response to energy imbalance resulting from restricted energy intake, plasma leptin response to exercise is more controversial. Following an acute bout of exercise, plasma leptin levels have been reported to be decreased (19, 41, 55, 56), unchanged (18, 35, 53, 78, 80) or increased (27). Activities such as 30-60 min of cycling exercise (53) and a 20-mile treadmill run (78) did not change leptin levels. However, other studies noted that a 2 hr run at 75% VO_{2max} (20), a marathon run (56) and an ultramarathon run (55) significantly decreased plasma leptin levels. Studies that observe the decreased leptin levels following physical activity often involve exercise of longer durations at higher intensities, resulting in greater energy expenditure (55, 56). Evidence of this comes from Landt et al. (55), who detected a 32% reduction in plasma leptin after a 101mile ultramarathon but not after 2 hr of strenuous cycling. In addition, Karamouzis et al. (41) reported a 50.5% reduction in leptin levels after marathon swimming (25 km sea swimming, 6.5-10.5 hr). These data suggest that higher exercise energy expenditures resulting in higher negative energy balance are more likely to alter leptin levels.

Although a greater exercise-induced negative energy balance would more likely cause a reduction in plasma leptin, controversial issues remain. Studies which observe decreased leptin levels following exercise either involve extremely high energy expenditure (41, 55, 56) or have obtained plasma samples during the 2 hr - 48 hr of recovery period (20, 23, 96) as opposed to immediately following the exercise bout (18, 35). Following exercise bouts of similar duration and intensity, Duclos et al. (20) detected a 30% reduction in plasma leptin 2 hr after the cessation of the exercise whereas Hickey et al. (35) found no difference when samples were taken immediately following

exercise. Additionally, Tuominen et al (96) reported a 34% reduction in plasma leptin levels 44 hrs after 2hr of running at 75% VO_{2max}, and Essig et al. (23) reported a 30% reduction in plasma leptin levels 48 hr after two treadmill exercise sessions with different energy expenditures (800 or 1,500 kcal) at 70% VO_{2max}.

These discrepancies may be partly explained by the response of other hormones to intense and prolonged exercise (13, 19, 47, 48, 61, 71). Counterregulatory hormones are critical for the maintenance of glucose homeostasis during exercise (13, 61, 88). During intense exercise (above 80% VO_{2max}), hepatic glucose production increases up to 8-fold while plasma catecholamines may increase up to 15-fold (61). Plasma insulin may remain constant or decrease slightly, whereas glucagon and cortisol will increase 2- and 3- fold, respectively (13, 61, 71, 88). During low to moderate intensity exercise, plasma epinephrine and norepinephrine increase to a lesser extent whereas cortisol does not increase substantially (47, 48). Cortisol is known to increase leptin production, while catecholamines are known to reduce plasma leptin production (5, 79, 86, 93). Also, a recent investigation demonstrated that the treatment of rat adipocytes with insulin increased tissue leptin content and secretion during a 2 hr incubation period (69). Therefore, if samples are collected during and immediately after exercise bouts, the net effect on leptin's response to exercise may be influenced by other hormones. As a result, changes in plasma leptin levels may not be observed.

There remains controversy over whether exercise training influences resting plasma leptin levels (31, 50, 54, 73, 76). However, most studies, which report a weight loss after exercise training, have also observed a reduction in resting plasma leptin levels (31, 50, 73). Halle et al. (31) studied resting plasma leptin levels in 20 men with type 2 diabetes mellitus (DM) before and after 6 weeks of training (2,200kcal expenditure/wk). Their exercise program caused a 4.3% weight loss and 30% reduction in plasma leptin level. Kohrt et al. (50) also reported a 4.9% weight loss and a 27.9% reduction in resting plasma leptin levels after 9 months of training. These results are not unexpected, as leptin is known to be produced and secreted by adipose tissue and it is well documented that plasma leptin correlates with body fat mass (FM) (16, 60). Although there is convincing evidence that exercise training, which leads to weight loss will decrease

plasma leptin level, it is unclear whether exercise training-induced leptin reduction is independent of changes in FM.

The sympathetic nervous system (SNS) plays an important role in leptin synthesis (15, 29, 38, 79, 85) and leptin -mediated resting metabolic rate (RMR) regulation (21, 38, 66), glucose uptake (33) and insulin secretion (65). Complete spinal cord lesions result in pathophysiological changes, which include: 1) loss of supraspinal regulatory control (47, 82), 2) reduced sympathetic activity (38, 67), and 3) morphologic changes in the sympathetic preganglionic neurons (52). As a result, hormonal and metabolic responses to a metabolic challenge have been altered in people with SCI, especially in people with cervical and high level thoracic SCI (47, 62, 75). However, the effects of exercise training on plasma leptin levels in people with SCI have not yet been investigated. Therefore, the purpose of the study is 1) to determine the effects of 12 weeks of FES-Rowing training on plasma leptin level in people with SCI and, 2) to investigate whether or not exercise training induced plasma leptin change in subjects with SCI is independent of fat mass change.

Research question:

1) Does training with FES-Rowing decrease plasma leptin levels in people with SCI?
2) Is the reduction in plasma leptin after training independent of FM change?

Research Hypothesis:

1) 12 weeks of FES-Rowing training decreases plasma leptin levels in people with SCI.

METHODS

The current study was part of a training study, which also determined the effects of FES-Rowing training on blood lipids in people with SCI.

Subject

Six healthy male subjects with paraplegia participated in the study (age 48.6±6, weight 70.06±3.28, injury levels between T4-T5). An institutional ethics review board at University of Alberta approved this study. All subjects gave their written informed consent to participate in the study. Individuals with pacemaker implants, uncontrolled arrhythmias, uncontrolled angina, congestive heart failure, current deep venous thrombosis, severe skin reaction to surface electrodes or FES, less than 90° of flexion at hips and knees, severe lower extremity spasticity, severe type 2 DM, and a regular aerobic exercise regime were excluded from the study. Subject characteristics are summarized in Table 1.

General study protocol:

After an initial interview, medical examination, and aerobic fitness assessment, subjects underwent a leg-strengthening training phase (0-4 weeks). A leg-strengthening training phase was implemented since many people with SCI are unable to perform FES-Rowing. Blood samples were obtained before and after the leg-strengthening training phase. Subjects who were able to complete a 10-minute bout of leg extension and flexion exercise proceeded to FES-Rowing training without the leg-strengthening training phase. Final blood samples were taken 48 hr after the last bout of exercise training in order to eliminate the acute effect of the last bout of exercise. Body composition and body weight were measured before any exercise training and within six days of the last bout of exercise training.

FES-Rowing VO_{2peak} testing:

Arm-only power output max (AMPO) was determined before the FES-Rowing $VO_{2\,peak}$ test. The AMPO test provides guidelines for setting the power output during VO_2peak testing. To determine AMPO, subjects were asked to start arm-only rowing at

10 watts and asked to increase the intensity of exercise by 10 watts at two-minute intervals until subjects voluntarily ceased the test due to exhaustion. The maximal power output that they were able to maintain for one minute was used as the reference value for the FES-Rowing $VO_{2\,peak}$ testing. Power output was monitored and recorded as displayed on the Concept II rowing machine (Morrisville, VT, USA).

Expired gases were assessed during the exercise testing using a Horizon metabolic measurement cart that provided data averaged over 15 seconds interval (Beckman, Sensormedics, Anaheim, CA, USA) and heart rate was monitored every minute using a Polar heart rate monitor (Woodbury NY, USA). To determine $VO_{2\,peak}$, subjects were asked to perform arm-only rowing without FES-stimulation at a comfortable rate (between 25 to 30 strokes per minute) for two minutes at 40% of their pre-determined AMPO. After the initial two minutes of arm -only rowing, subjects were asked to row another 2 min at 60% of AMPO with passive leg movement (the seat was manoeuvred by the research assistant). Then, subjects were asked to perform FES-Rowing with both upper and lower body muscle contraction with FES at a stroke rate between 25 to 30 strokes per minute at 80% of AMPO for two minutes. Thereafter, the subjects were asked to row at maximal power output until exhaustion. Criteria for a valid $VO_{2\,peak}$ test includes any two of the following: (17, 94): 1) respiratory exchange ratio greater than 1.1, 2) achievement of age-predicted maximum heart rate within 15 beats per minute (102), 3) maintenance or reduction of VO_2 despite an increase in workload. All subjects achieved an RER>1.1 during the $VO_{2\,peak}$ test.

Exercise training:

Subjects who were not able to complete 10-minutes of leg extension and flexion exercise against gravity were required to perform leg-strengthening training (75 leg extensions and flexions in 30 minutes, Spectrastim 2000, Therapeutic Alliance, Fairborn, OH) before they started training with FES-Rowing.

Pre- Rowing Training Criteria: 1) When the subjects were able to complete a 10-minute leg extension and flexion continuous exercise bout, the subjects proceeded to the 12 weeks of FES-Rowing training program. 2) If the subjects were not able to complete 10-minutes of leg extension and flexion exercise after two weeks of training, the leg-

strengthening phase was extended until the subjects were able to meet this criteria. Subject #1 and #3 completed six and seven sessions of leg-strengthening training, respectively. The other subjects were able to complete 10-minutes of leg extension and flexion exercise without leg-strengthening training.

FES-Rowing Machine: The FES-Rowing machine operates on an open-loop system and is suitable for use by persons with paraplegia. A specifically designed and modified electric stimulator (15 channel capability Motorola 68332 microcontroller with up to 15 mA and 180V, rectangular monophasic waveform, pulse with 0-500 μs) was mounted beneath a specially designed extended tract seating system for convenience and simple client-based electrode application (102). The seating system has been specially molded to include two adjustable backrests with a specialized honeycomb seat cushion and customized and fully adjustable seat belt system. Control of the extension and flexion phase of the rowing stroke was implemented manually through an open loop control system (simple, thumb-operated variable pressure sensors mounted on the rowing handle). Electronic data displayed by the rowing ergometer included strokes per minute, power output per stroke, and average power output for duration of the exercise bout.

FES-Rowing Training: Subjects trained for 12 weeks, 3-4 days per week. Initial sessions were conducted using sets of 5 minutes of rowing at 80% of predetermined FES-Rowing VO_{2peak} with a work to rest ratio of 1:0.5 (e.g. row 5 minutes, rest 2.5 minutes). Participants who were unable to row continuously for five minutes at the outset of the study were allowed 30-second rest intervals (e.g. If subject fatigued at three minutes, a 30-second rest was allowed, and then they were asked to continue rowing for two more minutes to complete a five-minute exercise interval). Participants were asked to expend 200 kcal per day or 600-800 kcal per week. This daily exercise-energy expenditure reflects an upper limit of exercise tolerance for SCI individuals. Individuals were unable to complete exercise bouts of greater value (higher intensity and/or longer duration). Power output and energy expenditure were monitored continually and adjusted on a biweekly basis. In order to monitor exercise intensity and energy expenditure, subjects were asked to perform FES-Rowing at the prescribed power output for five minutes and the steady state VO_2 was determined. If the steady state VO_2 was lower than 80% of

their most recently measured VO_{2peak} at the prescribed power output, the power output was increased by 10 W and then the steady state VO_2 was determined again. Based on the VO_2 and RER value at the prescribed power output, the energy expenditure and the time needed to expend 200 kcal were calculated (63).

Body Composition.

Pre- and post training body compositions were measured by dual-energy X-ray absorptiometry (DEXA) in all subjects. DEXA has been suggested as a practical and accurate way to measure body composition in people with SCI and able-bodied subjects (85). A CV of 3.8% is reported in the literature for FM assessment by DEXA (32). Subjects were asked to consume their usual diet and daily routine before the test. Subjects were asked to lie on a padded exam table, relax and breathe normally. FM, lean mass and percent body fat were measured.

Blood collection

Two or three blood samples were drawn (before and/or after leg-strengthening training and after completion of 12 weeks of FES-Rowing training). All blood samples were drawn from an antecubital vein after overnight fasting between 8:00 to 10:00am. For each sample, 21 ml of blood were drawn and collected in chilled vacutainers containing EDTA (norepinephrine assay) and heparin (insulin, leptin and glucose assay). Blood samples were separated by centrifugation at 4°C for 12 min at 3,000 rpm. Plasma was transferred to a storage vial and stored at -80°C until analyzed.

Analytical procedures

All plasma samples from each subject were analysed in duplicate in a single assay to eliminate between-assay variation. Plasma leptin (ng/dl) and insulin (μU/ml) were measured by RIA (human leptin RIA, human insulin RIA, Linco Research, Inc, St. Charles, MO) in our lab. The intra-assay coefficients of variation (CVs) were 3.4% and 6.1% respectively. Glucose levels were measured by the enzymatic method (Glucose Analyzer II, Beckman, Irvine, CA) in our lab and the intra-assay CV was 1.5% . Plasma norepinephrine (nmol/L) levels were measured by HPLC with electrochemical detection (electrochemical detector model 1045, Hewlett-Packard Co., Waldbronn, Germany at

the Calgary Laboratory Service in Foothills Hospital (Calgary, Alberta, Canada). The intra-assay CV for norepinephrine was 4.0%.

Statistical analysis:

The leg-strengthening phase was a period of adaptation; and used to determine baseline values. Therefore, pre and post leg-strengthening phase values were averaged and used as the pre-rowing training baseline. Since the data were not normally distributed, nonparametric statistics, Wilcoxon matched-paired signed ranks test was used to determine the effects of FES-Rowing training on each variable (95). Spearman's rank order correlation coefficients were used to test for the relationship between plasma leptin and body composition. The differences were considered significant at $P<0.05$. To determine the effects of FM change on plasma leptin levels after FES-Rowing training, the subjects were divided into two groups based on FM response to training: 1) FM loss group (> 1% loss of FM, Reseland et al. (81) report that as little as 1% FM reduction leads to a 4.1% reduction in circulating leptin), 2) FM maintained group (FM maintained or increased). Due to the small number of subjects, descriptive statistics were also used to describe the results from body composition, metabolic, hormonal response to 12 weeks of FES-Rowing training. All data are presented as means ± S.E.M.

RESULTS

Subjects

Table 2 illustrates subject characteristics before and after the 12 weeks of FES-Rowing raining. Six subjects completed the training program. However, the data from one subject was removed from group data analysis due to lack of adherence to the training program. The data for this subject are presented in Appendix A.

Exercise training

Exercise training data are summarized in Table 3. Subjects expended an average estimate of 199±16 kcal per training session for 36±3 exercise sessions. Over 12 weeks, they expended a total of 7079±546 kcal during exercise training. Exercise power output increased significantly after the exercise training (pre: 35.8±.6 vs post: 55.9±4.2 watts, P=0.043). VO_{2peak} did not change significantly after training (P=0.47)

Body composition

Group analysis (N=5): Body composition did not change significantly with training (P=0.14). Body composition data are summarized in Table 2 and Figure 1.

Descriptive analysis (N=5): FM decreased in three subjects (average 7.4% reduction) and remained unchanged in two subjects (average 1.6% increment) Lean mass increased in three subjects and remained unchanged in two subjects.

Metabolic and hormonal response to 12 weeks of FES-rowing training

Metabolic and hormonal response to training is also presented as FM loss vs FM maintained groups. Metabolic and hormonal responses to training are summarized in Tables 2 and 4, Figure 2, 3, and 4).

Group analysis (N=5): Plasma leptin was not significantly changed with 12 weeks of training (P=0.078). Plasma insulin (Figure 4) and norepinephrine did not change while plasma glucose (Figure 3) significantly decreased after the training (P=0.043). Also, plasma leptin and FM were positively correlated at both pre-(r=.90, P=0.04) and post-training (r=.90, P=0.037) periods (Figure 5).

Descriptive analysis: The data are summarized in Table 2, 4 and Figure 2, 3 and 4. The data are presented as 1) FM loss and maintained groups combined (N=5), 2) FM loss group (N=3) and 3) FM maintained group (N=2).

1) FM loss and FM maintained groups combined (N=5): Plasma leptin decreased by 33% with 12 weeks of training. Plasma leptin decreased in four out of five participants. Plasma glucose levels decreased in all five subjects (4.1%). Plasma insulin levels were reduced in four subjects (9.33%) and increased in one subject (13.66%). Plasma norepinphrine levels were reduced after training (2.52%).

2) FM loss group (N=3): An average FM loss in these subjects was 1.47 kg (7.43%). Plasma leptin levels were reduced by an average of 36%in all three subjects who lost FM. Plasma glucose levels were reduced in all three subjects by 11%. Plasma insulin levels were increased by 3.53% and norepinephrine levels were decreased by 9%.

3) FM maintained group (N=2): Plasma leptin levels were decreased in one subject (32.7%) but increased in the other subject (12%). There was a slight reduction in plasma glucose (1.5%) and insulin levels (11.7%) in both FM maintained subjects after the training. Norepinephrine levels increased in one subject by 30% and decreased in the other subject by 9.5%.

DISCUSSION

Although there are many studies that investigate the effects of exercise training on plasma leptin concentrations, to date there is no study that has investigated the effects of exercise training on plasma leptin in people with SCI. The purpose of the study was to: (a) to determine the effects of 12 weeks of FES-Rowing training in people with SCI whose injury levels were higher than T6 and (b) to explore whether reduction in plasma leptin after FES-Rowing training in people with SCI is independent of fat mass change.

The results of the present study did not show statistically significant changes to plasma leptin after 12 weeks of exercise training with FES-rowing in people with SCI (Table 4). However, descriptive statistics show that plasma leptin was reduced by 33% after the training (N=5). Plasma leptin levels were reduced in four out of five subjects after training. Considering that the effect size of plasma leptin levels after the training was 0.85, the primary mechanism for not finding statistical significance in plasma leptin after the training was likely the small sample size (N=5) leading to a lack of statistical power. The reduced exercise capacity in people with SCI may also explain the lack of change in plasma leptin levels after training.. In the current study, 200 kcal of exercise-induced energy expenditure was the upper limit of exercise tolerance for the volunteers, which is considerably less compared to an AB population. The SCI population are less capable of performing prolonged exercise at higher intensities compared to their AB counterparts. In addition, the literature suggests that approximately an 800kcal deficit is required to reduce plasma leptin levels following an acute bout of exercise (53).

There is considerable variation in the reported effects of exercise training on circulating leptin levels (31, 50, 54, 73, 76, 78). Studies have demonstrated a reduction (31, 50, 73), or no change (54, 78), in plasma leptin following an exercise-training program. The differences in these findings could be explained by concurrent weight loss with exercise training (31, 76). When weight loss occurs with training, reduction in plasma leptin levels are observed (31, 50, 73). Halle et al. (31) studied plasma leptin levels in 20 men with type 2 DM before and after six weeks of training (2,200kcal/wk). They reported that their exercise program caused a 4.3% weight loss and a 30% reduction in plasma leptin levels ($P<0.001$). Kohrt et al. (50) also reported a 4.9% loss in

body mass and a 27.9% reduction in plasma leptin levels after nine months of training ($P<0.01$). In the current study, we observed that a 1.9% reduction in body weight and a 4.6% reduction in FM result in 33.1% reduction in plasma leptin levels ($P=0.078$). In addition, those subjects who lost FM after training also showed reduced plasma leptin levels. These results are not unexpected, as leptin is known to be produced and secreted by adipose tissue, and it is well documented that plasma leptin correlates with FM (16, 60). Plasma leptin levels correlated with fat mass before and after training in the current study. Also, we have recently demonstrated that plasma leptin levels correlate with fat mass in people with tetraplegia (38).

Although there is convincing evidence that exercise training that leads to weight loss will decrease plasma leptin levels, it is unclear whether exercise training-induced leptin reduction is solely dependent on changes in FM. We have divided our subjects into two subgroups: FM loss and FM maintained group. Fat mass was reduced in three subjects while FM of two subjects remained unchanged or slightly increased. Plasma leptin was reduced by 36% and 23% in FM loss and FM maintained group, respectively. Although plasma leptin was reduced more in the FM loss group, the FM maintained group also showed reduced plasma leptin after the training. This may suggest that another mechanism is contributing to reduced plasma leptin levels. Hickey et al. (36) reported a reduction in plasma leptin levels in females after 12 weeks of training (4d/wk, 1200 kcal/wk) despite stable fat mass. Pasman et al. (76) also reported a reduction in plasma leptin levels in obese males after 10 months of exercise training, independent of changes in body fat mass ($P<0.05$). Furthermore, Okazaki et al. (73) demonstrated that plasma leptin levels decreased in 14 subjects whose fat mass either did not change or slightly increased after a 9 month exercise intervention ($P<0.05$). Moreover, Ishii et al. (35) report a reduction in plasma leptin with the combination of diet and exercise, but not with diet alone after 6 weeks of exercise training. Ishii et al. (37) expressed leptin levels per kg fat mass and found that plasma leptin per kg fat mass was lower in the combined diet and exercise group compared to the diet only group ($p<0.05$). Therefore, these authors suggest that exercise training can reduce plasma leptin levels independent of fat mass loss.

Knowing that exercise training may reduce plasma leptin levels independent of FM changes, repetitive exercise stimulation of β_3-Adrenergic stimulation may contribute to reduced plasma leptin levels after the training (7, 24, 74). Bramlett et al. (7) tested the hypothesis that β_3-adrenergic receptor stimulation was involved in the downregulation of leptin mRNA levels. Rats were randomised into one of four groups: control, control +β_3 antagonist, 55 min of treadmill running exercise alone and 55 min of treadmill running exercise +β_3 antagonist (SR-59230A). They found that exercise alone reduced leptin mRNA in retroperitoneal fat, but β_3 antagonism blocked this effect. Interestingly, intense exercise increases norepinephrine levels up to 15 times (13, 61, 88). Knowing that norepinephrine infusion acutely decreases both ob gene expression and plasma leptin levels (15, 79), it is assumed that a regulative pathway involving the suppression of leptin release during exercise may be mediated by sympathetic activation of adipose adrenergic receptors. However, people with high lesion SCI lost their ability to increase catecholamine secretion in response to exercise as well as hypoglycemia (28, 47, 82). Catecholamine and lactate responses to an incremental VO2 peak test were measured in three men with SCI above T6 and four men with SCI below T6. Subjects with a lower level injury showed a higher epinephrine (Low vs High: 0.5±0.2 vs 0.1 ng/ml), norepinephrine (3.1±1.0 vs 0.48±0.11 ng/ml) and lactate (7.1±0.5vs 3.2±0.4 mM) response compared to subjects with a higher-level injury (28). Palmer et al. (75) investigated the catecholamine response to insulin-induced hypoglycemia in able-bodied (AB) controls and in subjects with high lesion SCI and AB subjects. They found that catecholamine levels did not increase in people with high lesion SCI while it increased more than 200% in AB controls. This result was confirmed by another study (62), in which a 12.8 fold increase in norepinephrine during insulin-induced hypoglycemia in AB controls was reported, while no change was observed in the high lesion SCI group. Therefore, a blunted catecholamine response to exercise and the SNS impairment in people with high lesion SCI in the current study likely eliminated the possibility of SNS-mediated leptin reduction after exercise training.

If exercise-induced leptin reduction in the current study is not mediated by the SNS nor fat mass changes, it may be related to changes in skeletal muscle after the training. There are a total of five spliced forms of leptin receptors: Ob-Ra, Ob-Rb, Ob-Rc,

Ob-Rd and Ob-Re (1, 4). The receptors share an identical extracellular ligand-binding domain of 840 amino acids at the amino terminus as well as a transmembrane domain of 34 amino acids (1, 4). The Ob-Rb is the only isoform with all the protein motifs: an extracellular domain, capable of binding to leptin; a short transmembrane region; and a cytoplasmic region, capable of signal transduction (1). It has been hypothesized that Ob-Rb was located only in hypothalamus. However, recent evidence suggests that peripheral organs including skeletal muscle and adipose tissue also express OB-Rb (1). This fact raises a question as to whether or not leptin may exert its action directly at the level of the target tissue. Minokoshi et al. (64) provided convincing evidence that leptin stimulates adenosine 5'-monophosphate-activated protein kinase (AMPK) activity directly in skeletal muscle as well as via the SNS. AMPK regulates lipid oxidation in muscle by reducing acetyl-CoA carboxylase (ACC) activity, which in turn decreases malonyl-CoA levels and increases the mitochondrial uptake of free fatty acid (FFA). They demonstrated that an intravenous injection of leptin increases AMPK activity in skeletal muscle at 15 min and at 6 hours after injection. To determine whether the effects of leptin on AMPK activation require the hypothalamic-SNS axis, they used pharmacological adrenergic blockade and two kinds of surgical denervation. Denervation blocked the ability of intrahypothalamic leptin or intravenous leptin 6 hrs after injection to stimulate alpha 2 AMPK in the soleus muscle. However, alpha2 AMPK activation at 15 min after intravenous leptin injection remained intact in denervated muscle. This study demonstrated that leptin exerts its effect directly to the skeletal muscle within 15 min from an intravenous leptin injection, whereas, it takes three to six hours for a SNS-mediated leptin affect to increase AMPK activity (64). These findings suggest that leptin has peripheral effects on skeletal muscle by directly stimulating AMPK activity.

Barzilai et al. (3) found that following 8 days of leptin treatment in Spraque-Dawley rats, whole body glucose uptake as assessed by the euglycemic clamp technique was increased 52% compared with control animals. Since over 80% of the glucose load is disposed by skeletal muscle, it is possible that leptin may be capable of acting on skeletal muscle to improve insulin action (3, 40). Burks et al. (8) demonstrated that mice lacking insulin receptor substrate (IRS)-2 have a five-fold increase in leptin levels by

eight weeks old and that IRS-2 plays an important role in the leptin signaling pathway which involves signal transducers and activators of transcription (STAT)-3 phosphorylation. In addition, Kim et al. (45) recently demonstrated that three min after intravenous leptin injection in normal rats, there is an increased phosphorylation of STAT3 and STAT1 in adipose tissue and phosphorylation of mitogen-activated protein kinase (MAPK) in adipose tissue and liver. Also, they found that IRS-1 associated phosphatidylinositol-3-OH kinase (PI3-K) activity in adipose tissue and IRS-2-associated PI3-K activity in liver were moderately increased in leptin-injected rats. Moreover, Niswender et al. (72) observed the effect of systemically administered leptin on the activity of PI3-K and STAT3 in male Wister rats and demonstrated that an infusion of PI3-K inhibitor (LY294002) completely blocks leptin's effects on food intake. These studies suggest the existence of cross-talk between insulin and leptin receptor pathways (1, 3, 42, 45, 72). They also raise the question as to whether or not exercise could improve peripheral leptin sensitivity in a similar fashion to exercise-induced peripheral tissue insulin sensitivity.

Interestingly, exercise (acute and chronic exercise) is known to increase phosphorylation of PI3-K, MAPK, AMPK, and activity of IRS (10, 46, 57, 70). Chibalin et al. (10) reported that insulin-stimulated tyrosine phosphorylation of IRS-1 and associated PI3-K activity increased 2.5 and 3.5-fold after 1 and 5 days of exercise. After 1 day of exercise, IRS-2 protein expression increased 2.6 fold and basal and insulin-stimulated IRS-2 associated PI3-K activity increased 2.8 fold and 9 fold, respectively. Also, Kirwin et al. (46) reported that exercise-trained group had a significantly higher insulin activation of IRS-1-associated PI-3 kinase activation compared with the sedentary control group ($P<0.004$). Treadmill running and muscle contractions induced by electrical stimulation also improves MAPK and AMPK activities, which stimulate translocation of GLUT-4 (57, 70). Knowing that leptin increases glucose uptake through insulin-dependent as well as independent pathways such as IRS-2 expression, IRS-1 associated PI3-K activity, MAPK and AMPK activities, the exercise-induced improvement in these factors could improve the effectiveness of leptin on skeletal muscle (10, 46, 57, 70).

In addition, exercise training increases FFA binding proteins, mitochondrial density and enhances the rate of FFA oxidation in the skeletal muscle and thus decreases the level of intramuscular TG content. Stephens et al. (92) reported that there is a large, progressive increase in ACCβ phosphorylation during moderate-intensity exercise at 60% VO2peak in humans, which is coupled with the progressive increase in AMPKα2 activity and fat oxidation. Knowing that increased intramuscular TG content in obese individuals is the cause of skeletal muscle leptin resistance (90, 91, 104), it could be possible that an exercise-induced reduction in TG content may improve leptin sensitivity (91). It is also possible that aerobic exercise training could improve skeletal muscle leptin sensitivity and, consequently, decrease plasma leptin levels. If the effects of exercise on plasma leptin and leptin sensitivity follow the pattern of the effects of exercise on plasma insulin and insulin sensitivity (77), measuring plasma leptin levels without peripheral leptin sensitivity may not accurately represent the effect of exercise on leptin metabolism. Although we did not measure molecular and biochemical changes in skeletal muscle, we have previously demonstrated that exercise with FES results in positive changes in GLUT-1 and 4 content (11), number of capillaries per fiber, fiber types and other oxidative enzymes in people with SCI (12).

Just as improved insulin sensitivity would decrease plasma glucose and insulin levels (77), exercise-induced leptin sensitivity may reduce plasma leptin levels. Improvement in leptin sensitivity would increase effectiveness of leptin on fatty acid oxidation and glucose metabolism (90, 91). Acute leptin administration causes a repartitioning or shunting of fatty acid metabolism toward oxidation and away from esterification in skeletal muscle (90, 91, 104). Leptin also stimulates the hydrolysis of stored intramuscular TG (90). Hence, improved effectiveness of fatty acid oxidation will further decrease intramuscular TG. Unger (98) proposed and provided evidence that leptin is the principal hormone of liporegulation, maintaining normal intracellular lipid homeostasis despite wide variations in dietary fat. This resembles the way in which insulin maintains tolerance to wide variations in dietary carbohydrate intake. On a 60% fat diet, adipocyte fat content of normal rats increases almost 150-fold, whereas that of pancreatic islets, liver, heart, and skeletal muscle rises less than 10-fold. He proposed that this low level of TG deposition in non-adipocytes during the consumption of excess

dietary fat is the result of the release of leptin in proportion to ingested TG excess being stored in adipocytes (97). Therefore, if leptin is deficient, or if its target tissues become unresponsive during positive energy balance, steatosis will occur (97). This leads ultimately to so-called lipotoxicity, dysfunction of nonadipose tissue such as the pancreatic β-cells, and skeletal muscle and may culminate in fatty acid-induced apoptosis (97, 98). The net result of lipotoxic disorders in humans is called "syndrome X". As aerobic exercise is known to decrease TG accumulation in the skeletal muscle, improvement in leptin sensitivity may decrease the risk of lipotoxicity and eventually decrease the risk of developing hyperlipidemia, cardiomyopathy, insulin resistance and type 2 diabetes mellitus (DM), the familiar components of the "syndrome X".

In the current study, we observed a significant decrease in plasma glucose levels after training ($P<.05$). Studies suggest that glucose metabolism is partly responsible for leptin regulation (64, 95). The importance of glucose metabolism in regulating the level of leptin was demonstrated by Boden et al. (6) who found that glucose infusion prevented the fasting-induced leptin decline. Also, Mueller et al (68). investigated the effects of inhibitors of glucose transport and metabolism on leptin secretion from rat adipocytes. They found that leptin secretion was closely related to glucose metabolism ($r=0.64$, $P<0.0001$). Wang et al. (99) suggested that glucoreglation of leptin production may be mediated by the entry of glucose in the glucosamine pathway via L-glutamine the D-fructose-6-phosphate amidotransferase step.

Also, insulin sensitivity influences entry of glucose into the cell (58, 77). Although we did not measure insulin sensitivity in our study, we have demonstrated previously that eight weeks of exercise with FES cycling improves glucose tolerance and insulin sensitivity in people with SCI (40). Exercise intensity and duration in the current study were much greater than the Jeon et al (40) study and therefore improved insulin sensitivity in the current study would be expected. Segal et al (84). report that insulin resistance is associated with an elevated plasma leptin concentration in males. It is thus conceivable that the reduction in insulin resistance accompanied with reduction in plasma glucose may have decreased plasma leptin levels in our study.

CONCLUSION:

To our knowledge, this is the first study to investigate the effects of exercise training on plasma leptin in people with SCI. Statistically, the change in plasma leptin was not significant (P=0.079); however, a trend toward a reduction in plasma leptin (33%) after the training was observed. The leptin reduction could be partly explained by a reduction in fat mass; however, it may also be due to decreased plasma glucose and peripheral tissue leptin sensitivity.

REFERENCE

1. Ahima RS, Flier JS. Leptin. Annu Rev Physiol. 62:413-437, 2000.
2. Ahima RS. Role of leptin in the neuroendocrine response to fasting. Nature 382: 250-252, 1996.
3. Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L. Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100:3105, 1997.
4. Bjorbaek Ch, Uotani S, Dilva B, Flier JS. Divergent signalling capacities of the long and short isoforms of the leptin receptor. J Biol Chem. 272:32686-32695, 1997.
5. Boden G, Chen X, Kolaczynski JW, Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest 100:1107-1113, 1997.
6. Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. J Clin Endo Met 81:3419-3423, 1996.
7. Bramlett SB, Zhou J, Harris RBS, Handry SL, Witt TL, Zachwieja JJ. Does beta3-adrenoreceptor blockade attenuate acute exercise-induced reductions in leptin mRNA? J Appl Physiol 87:1678-1683, 1999.
8. Burks DJ, de Mora JF, Schubert M, Withers DJ, Myers MG, Towery HH, Altamuro SL, Flint CL, White MF. IRS-2 pathways intergrate female reproduction and energy homeostasis. Nature. 407:377-382, 2000.
9. Chen G, Koyama K, Yuan X, et al. Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proc Natl Acad Sci USA. 93:14795-14799, 1996.
10. Chibalin AV, Yu M, Ryder JW, Song XM, Galuska D, Krook A, Wallberg-Henriksson H, Zierath JR. Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: Differential effects on insulin-receptor substrates 1 and 2. Proc Natl Acad Sci USA 97:38-43, 2000.
11. Chilibeck PD, Bell G, Jeon JY, Weiss CB, Burnham RS. Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. *Metabolism* 48: 1409-1413, 1999.

12. Chilibeck PD, Jeon JY, Weiss CB, Bell G, Burnham RS. Histochemical changes in muscle of individuals with spinal cord injury following functional electrical stimulated exercise training. *Spinal Cord* 37:264-268, 1999.

13. Coker RH, Krishna MG, Lacy DB, Bracy DP, Wasserman DH. Role of hepatic α- and β-adrenergic receptor stimulation on hepatic glucose production during heavy exercise. Am J Physiol. 273:E831-E838, 1997.

14. Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS. Role of leptin in fat regulation. Nature 380:677, 1996.

15. Commins S, Marsh D, Thomas S et al. Norepinephrine is required for leptin effects on gene expression in brown and white adipose tissue. Endocrinology. 140: 4772-4778, 1999.

16. Considine RV. Sinha MK. Heiman ML. Kriaugiunas A. Stephens TW. Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL. Serum immunoreactive-Leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334:292-295, 1996.

17. Crouse SF, O'Brien BC, Grandjean PW, Lowe RC, Rohack JJ, Green JS. Effects of training and a single session of exercise on lipids and apolipoproteins in hypercholesterolemic men. J Appl Phyiol 83:2019-2028, 1997.

18. Dirlewanger M, Vetta VD, Giusti V, Schneiter P, Jequier E. Tappy L. Effect of moderate physical activity on plasma leptin concentration in humans. Eup J Appl Physiol. 79:331-335, 1999.

19. Doucet E, ST-Pierre S, Almeras N, Mauriege P, Despres JP, et al. Fasting insulin levels influence plasma leptin levels independently from the contribution of adiposity: Evidence from both a cross-sectional and an intervention study. J Clin Endocrinol Metab. 85:4231-4237, 2000.

20. Duclos M, Corcuff JB, Ruffie A. Roger P, Manier G. Rapid leptin decrease in immediate post-exercise recovery. Clin Endocrinol 50:337-342, 1999.

21. Dunbar JC, Hu Y, Lu H. 1997 Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. Diabetes 46:2040-2043.

22. Elias CF, Aschkenasi C, Lee, C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK. Leptin differentially regulate NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23: 775-786, 1999.

23. Essig DA, Alderson NL, Ferguson MA. Bartoli WP, Durstine JL. Delayed effects of exercise on the plasma leptin concentration. Metabolism 49:359-399, 2001.

24. Evan BA, Agar L, Summers RJ. The role of the sympathetic nervous system in the regulation of leptin synthesis in C57BL/6 mice. FEBS Lett 444:149-154, 1999.

25. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetam CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effets of recombinant leptin therapy in a child with congenital leptin deficiency. New Eng J Med 341:879-884, 1999.

26. Fei H, Okano HJ, Li C. et al. Anatomic localization of alternatively spliced leptin receptor (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci USA. 94:7001-7005, 1997.

27. Fisher JS, Van Pelt RE, Zinder O. Landt M, Kohrt WM. Acute exercise effect on postabsorptive serum leptin. J Appl Physiol. 91:680-686, 2001.

28. Frey GC, McCubbin JA, Dunn JM, Mazzeo RS. Plasma catecholamine and lactate relationship during graded exercise in men with spinal cord injury. Med Sci Sports Exerc. 29:451-456, 1996.

29. Getty TW, Harkness PJ, Watson PM. The β_3 adrenergic receptor inhibits insulin-stimulated leptin secretion form isolated rat adipocytes. Endocrinology 137: 4054-4057, 1996.

30. Halaas JL, Gajiwala KS, Meffei M, Cohen SL, Chait BT. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546, 1995.

31. Halle M, Berg A, Garwers U, Grathwohl D, Knisel W. Keul J. Concurrent reductions of serum leptin and lipids during weight loss in obese men with type II diabetes. Am J Physiol. 277:E277-E282, 1999.

32. Hansen NJ, Lohman TG, Going SB, Hall MC, Pamenter RW, Bare LA, Boyden TW, Houtkooper LB. Prediction of body composition in pre-menopausal females from dual-energy x-ray absorptiometry. J Appl Physiol, 75: 1637-1641, 1993

33. Haque MS, Minokoshi Y, Hamai M, Iwai H, Horiuchi M, Shimazu T. Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral

tissues after intrahypothalamic injection of leptin in rats. Diabetes. 48:176-1712, 1999.

34. Harvey G. Regulation of energy balance. Nature 222:629-631, 1969.
35. Hickey MS, Considine RV, Israel RG, Mahar TL, McCammon MR, Tyndall GL, Houmard JA, Caro JF. Leptin is related to body fat content in male distance runners. Am J Physiol: E938-E940, 1996.
36. Hickey MS, Houmard JA, Considine RV, Tyndall GL, Midgette JB, Gavigan KE, Weidner ML, McCammon MR, Israel RG, Caro JF Gender-dependent effects of exercise training on serum leptin levels in humans. Am J Physiol Endocrinol Metab. 272:E562-566, 1997.
37. Ishii T, Yamakita T, Yamagami K, Yamamoto T, Miyamoto M, Kawasaki K, Hosoi M, Yoshioka K, Sata T, Tanaka S, Fujii S. Effect of exercise training on serum leptin levels in type 2 diabetic patients. Metabolism 50:1136-1140, 2001.
38. Jeon YJ, Harber VJ, Bell G, McCargar L, Steadward RD, Wheeler GD. Intact Sympathetic Nervous System is Required for Leptin Effects on Resting Metabolic Rate in People with Spinal Cord Injury. J Clin Endocri Metab 88:402-407, 2003.
39. Jeon YJ, Harber VJ, Steadward RD. Leptin response to short term fasting in sympathectomized men- role of sympathetic nervous system. American Journal of Physiology: Endocrinology and Metabolism 284:E634-E640, 2003.
40. Jeon YJ, Weiss BC, Steadward RD, Ryan E, Burnham R, Bell G, Chilibeck P, Wheeler GD. Improved glucose tolerance and insulin sensitivity after electrical stimulation-assisted cycling in people with spinal cord injury, Spinal Cord 40:110-117, 2002
41. Karamouzis I, Karamouzis M, Vrabas IS, Christoulas K, Kyriazis N, Giannoulis E, Mandroukas K. The effects of marathon swimming on serum leptin and plasma neuropeptide Y levels. Clin Chem Lab Med 40:132-136, 2002.
42. Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, Haring HU. Leptin activates PI3-Kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. Diabetologia 40:1358-1362, 1997.
43. Kennedy G. The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Sco London 140:578-592, 1953.

44. Krassioukov AV, Bunge RP, Pucket WR, Bygrave MA. The changes in human spinal sympathetic preganglionic neurons after spinal cord injury. Spinal Cord 37:6-14, 1999

45. Kim YB, Uotani S, Pierroz DD, Flier JS, Kahn BB. In Vivo administration of leptin activates signal transduction directly in insulin-sensitive tissues: overlapping but distinct pathways from insulin. Endocrinology 141:2328-2339, 2000.

46. Kirwan JP, del Aguila LF, Hernandez JM, Williamson DL, O'Gorman DJ, Lewis R, Krishnan RK. Regular exercise enhances insulin activation of IRS-1-associated PI3-kinase in human skeletal muscle. J Appl Physiol 88:797-803, 2000.

47. Kjaer M Pollack SF, Mohr T. et al. Regulation of glucose turnover and hormonal responses during electrical cycling in tetraplegic humans. Am J Physiol 271:R191-R199, 1996.

48. Kjaer M, Dela F, Sorensen FB. et al. Fatty acid kinetics and carbohydrate metabolism during electrical exercise in spinal cord injured human. Am J Physiol. 281:R1492-R1498, 2001.

49. Klein S. Horowitz JF. Landt M. Goodrick SJ. Mohamed-Ali V. et al. Leptin production during early starvation in lean and obese women. Am J physiol Endocrinol Metab. 278: E280-E284, 2000.

50. Kohrt WM, Landt M, Birge SJ. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older females. J Clin Endocrinol Metab 81:3980-3985, 1996.

51. Kolaczynski JW, Nyce MR, Considine RV et al. Acute and chronic effects of insulin on leptin production in humans: studies on vivo and in vitro. Diabetes 45:699-701, 1996.

52. Krassioukov AV, Bunge RP, Pucket WR, Bygrave MA. The changes in human spinal sympathetic preganglionic neurons after spinal cord injury. Spinal Cord. 37:6-13, 1999.

53. Kreamer RR, Johnson LG, Haltom R, Kraemer GR, Hebert EP. Gimpel T, Castracane VD. Serum leptin concentrations in response to acute exercise in postmenopausal women with and without hormone replacement therapy. PSEBM 221:171-177, 1999.

54. Kreamer RR, Kreamer GR, Acevedo EO, Hebert EP, Temple E, Bates M, Etie A, Haltom R, Quinn S, Castracane VD. Effects of aerobic exercise on serum leptin levels in obese women. Eur J Appl Physiol 80:154-158, 1999.

55. Landt M, Lawson GM, Helgeson JM, Davila-Roman VG, Ladenson JH, Jaffe AS, Hickner RC. Prolonged exercise decreases serum leptin concentration. Metabolism 46:1109-1112, 1997.

56. Leal-Cerro A, Garcia-Luna RP, Astorga R, Parejo J, Peino R. Peino R, Dieguez C, Casanueva FF. Serum leptin levels in male marathon athletes before and after the marathon run. J Clin Endocrinol Metab. 83:2376-2379, 1998.

57. Lee JS, Bruce CR, Spurrel BE, Hawley JA. Effect of training on activation of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase pathways in rat soleus muscle Clin Exp Pharmacol Physiol. 29:655-660, 2002.

58. Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WG, Zawadzki JK Yki-Jarvinen, H, Chrisin L, Bogardus C. Skeletal muscle capillary density and fibre type are possible determinants of in vivo insulin resistance in men. J Clin Invest 80:415-424, 1987.

59. Maccario M, Aimaretti G, Gorneli G, Gauna C, Grottoli S. et al. Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans. Am J physiol Endocrinol Metab 279: E411-E416, 2000.

60. Mantzoros CS. Moschos S, Avramopoulos I, Kaklamani V, Liolios A, Doulgerakis DE, Griveas I, Katsilambros N, Flier JS. Leptin concentrations in relation to body mass index and the tumor necrosis factors-α system in humans. J Clin Endocrinol Metab 82:3408-3413, 1997.

61. Marliss EB, Simantirakis E, Miles PDG, Hunt R, Gougeon-Reyburn R et al. Glucose turnover and its regulation during intense exercise and recovery in normal male subjects. Clin Invest Med. 15:406-419, 1992.

62. Mathias CJ, Frankel HL, Turner RC, and Christensen NJ. Physiological responses to insulin hypoglycemia in spinal man. *Paraplegia* 17: 319-326, 1979-1980.

63. McArdle WD, Katch F, Katch V. Exercise physiology: energy, nutrition and human performance (4th edition). Williams & Wilkins p 147, 1996

64. Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415:339-343, 2002

65. Mizuno A, Murakami T, Otani S, Muwajima M, Shima K. Leptin affects pancreatic endocrine function through the sympathetic nervous system. Endocrinology 139: 3863-3870, 1998.

66. Monroe M, Seals D, Shapiro L et al. Direct evidence for tonic sympathetic nervous support of resting metabolic rate in healthy adult humans. Am J Physiol Endocrinol Metab. 280: E240-744, 2001.

67. Monroe MB, Tataranni PA, Pratlley R et al. Lower daily energy expenditure as measured by a respiratory chamber in subjects with spinal cord injury compared with control subjects. Am J Clin Nutr. 68:1223-1227, 1998.

68. Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, et al. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology. 139:551-558, 1998.

69. Murakami T, Iida M, Shima K. Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem Biophys Res Commun. 214:127-127, 1995.

70. Musi N, Hayashi T, Fujii N, Hirshman MF, Witters LA, Goodyear LJ. AMP-activated protein kinase activity and glucose uptake in rat skeletal muscle. Am J Physiol Endocrinol Metab 280:E677-E684, 2001.

71. Natali A, Gastaldelli A, Galvan AQ, Sironi AM, Ciociaro D. et al. Effects of acute β_2-blockade on insulin action and secretion in humans. Am J Physiol 274:E57-E64, 1998.

72. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myer Jr. MG, Schwartz MW. Key enzyme in leptin-induced anorexia. Nature 413:794-795, 2001.

73. Okazaki T, Himeno E, Nanri H. Ogata H, Ikeda M. Effects of mild aerobic exercise and a mild hypocaloric diet on plasma leptin in sedentary women. Clin Exp Pharm Physiol 26:415-420, 1999.

74. Pagano C, Marzolo M, Granzotto M, Ricquier D, Federspil G, Vettor R. Acute effects of exercise on circulating leptin in lean and genetically obese fa/fa rats. Biochem Biophysical Res Commu 255, 698-702, 1999.

75. Parmer JP, Henry DP, Benson JW, Johnson DG, Ensinck JW. Glucagon response to hypoglycemia in sympathectomized man. J Clin Invest 57: 522-525, 1976.

76. Pasman WJ, Sesterterp-Plantenga MS, Saris WHM. The effect of exercise training on leptin levels in obese males. Am J Physiol. 274:E280-E286, 1998.

77. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, Rothman DL, Shulman GI. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. New Eng J Med 335:1357-1362, 1996.

78. Perusse L, Collier G, Gagnon J. Leon AS, Rao DC, Skinner JS, Wilmore JH, Nadeau A, Zimmet PZ, Bouchard C. Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol. 83:5-10, 1997.

79. Pinkney JH, Coppack SW, Mohamed-Ali V. Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol 48:407-411, 1998.

80. Racette SB, Coppack SW, Landt M, Klein S. Leptin production during moderate-intensity aerobic exercise. J Clin Endocrinol Metab. 82:2275-2277, 1997.

81. Reseland JE, Anderssen SA, Solvoll K, Hjermann I, Urdal P, Holme I, Drevon CA. Effect of long-term changes in diet and exercise on plasma leptin concentrations. Am J Clin Nutr 73:240-5, 2001.

82. Schmid A, Huonker M, Barturen JM, Stahl F, Schmidt-Trucksass A. Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. J Appl Physiol. 85:635-641, 1998.

83. Schwartz MW, Seeley R, Campfield A, Burn P, Baskin DG. Identification of Targets of leptin action in rat hypothalamus. 98:1101-1106, 1996.

84. Segal KR, Landt M, Klein S. Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes. 45: 988-91, 1996.

85. Sivitz WI, Fink BD, Morgan DA. et al. Sympathetic inhibition, leptin, and uncoupling protein subtype expression in normal fasting rats. Am J Physiol. 277:E668-E677, 1999.

86. Solano JM, Jacobson L. Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am J Physiol Endocrinol Metab 277:E708-E716, 1999.

87. Sonnenberg GE, Krakower GR, Hoffmann RG, Maas DL, Hennes MMI, Kissebah AH. Plasma leptin concentration during extended fasting and graded glucose infusions: Relationships with changes in glucose, insulin and FFA. J Clin Endo Metabol. 86: 4895-4900, 2001.

88. Sotsky MJ, Shilo S, Shamoon H. Regulation of counterregulatory hormone secretion in man during exercise and hypoglycemia. J Clin Endocrinol Metabol. 68:9-16, 1989.

89. Spurgen AM, Bauman WA, Wang J, Person Jr. RN. Measurement of body fat in individuals with tetraplegia: a comparison of eight clinical methods. Paraplegia 33:402-408, 1995

90. Steinberg GR, Parolin ML, Heigenhauser GJF, Dick DJ. Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. Am J Physiol Endocrinol Metab 283:E187-E192, 2002.

91. Steinberg GR, Rush JW, Dyck DJ. AMPK expression and phosphorylation are increased in rodent muscle after chronic leptin treatment. Am J Physiol Endocrinol Metab 284:E648-654, 2003

92. Stephens TJ, Chen ZP, Canny BJ, Michell BJ, Kemp BE, McConell GK. Progressive increase in human skeletal muscle AMPKα2 activity and ACC phosphorylation during exercise. Am J Physiol Endocrinol Metab 282:E688-E694, 2002.

93. Tan JT, Patel BK, Kaplan LM, Keonig JI, Hooi SC. Regulation of leptin expression and secretion by corticosteroids and insulin-Implication for body weight. 8:85-92, 1998.

94. Taylor HL, Buskirk E, Henschel A. Maximal oxygen intake as an objective measure of cardio-respiratory performance. J Appl Physiol 8:73-80, 1995.

95. Thomas JR, Nelson JK. Research methods in physical activity (2nd edition). Human Kinetics, Campaign, Illinois, p 179-197, 1990.

96. Tuominen JA, Ebeling P, Laquier FW, Heiman ML, Stephens T. Koivisto VA. Serum leptin concentration and fuel homeostasis in healthy man. Eup J Clin Invest. 27:206-211, 1997.

97. Unger RH. Lipotoxic disease. Annu. Rev. Med. 53:319-336, 2002.

98. Unger RH, Zhou Y.T. Lipotoxicity of β-cells in obesity and in other causes of fatty acid spill over. Diabetes 50 (Suppl): S118-121, 2001.

99. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393: 684-688, 1998.

100. Weiss BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R, Gougeon R. Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentration in obese women. Am J Clin Nutri. 70:321-330, 1999.

101. Wellhoener P, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W. et al. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. J Clin Endocrinol Metab. 85:1267-1271, 2000.

102. Wheeler GD, Andrews B, Lederer R, Davoodi R, Natho K, Weiss C, Jeon J, Bhambhani Y, Steadward RD. Functional Electrical Stimulation-Assisted Rowing: Increasing Cardiovascular Fitness Through Functional Electric Stimulation Rowing Training in Persons with Spinal Cord Injury. Arch Phys Med Rehabil 83:1093-1099, 2002.

103. Yaspelkis BB, Ansari L, Ramey E, Holland GJ, Loy SF. Chronic leptin administration increases insulin mediated skeletal muscle glucose uptake and transport. Metabolism. 48:671-676, 1999.

104. Yaspelkis III BB, Davis JR, Saberi M, Smith TL, Jazayeri R, Singh M, Fernandes V, Trevino B, Chinookoswong N, Wang J, Shi ZQ, Levin N. Leptin administration improve skeletal muscle insulin responsiveness in diet-induced insulin resistance rats. Am J Physiol Endocrinol Metab, 280:E130-E142, 2001.Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positioning cloning of the mouse obese gene and its human homologue. Nature. 372:425-432, 1994.

105. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positioning cloinging of the mouse obese gene and its human homologue. Nature. 372:425-432, 1994.

Table 1. Subject Characteristics

Subject	Age (yr)	Height (cm)	Pre-wt (kg)	Post-wt (kg)	Years since injury (yr)	Injury level	ASIA class
1	49	180	76.4	75.1	21	T5	A
2	56	167.5	79.4	75.6	36	T4	A
3	24	180	64.5	65.5	1.5	T5	B
4	53	172.5	66.8	65.5	21	T4	A
5	47	162.5	63.2	62	31	T5	A
Average	48±6	172.5±3.4	70.1±3.3	68.7±2.8	22.1±6		

Data are the mean ± SE. ASIA: The International Standards for Neurologic and Functional Classification by American Spinal Cord Injury Association. A: Complete injury - No motor or sensory function is preserved in the sacral segments S4-5. B: Incomplete - Sensory but no motor function is preserved below the neurologic level and extends through the sacral segments S4-5.

Table 2. Physical, metabolic and hormonal parameters pre- and post-training in individual subjects.

	S1		S2		S3		S4		S5	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	76.4	75.1	79.4	75.6	64.5	65.5	66.8	65.5	63.2	62
FM (kg)	21.3	20.1	23.4	21.9	14.6	12.9	14.8	14.8	13.2	13.6
Lean mass (Kg)	51.5	51.7	52.8	52.6	47.6	49.7	45	48.5	58.6	58.1
Percent fat (%)	28.50	27.20	30.00	28.70	22.50	19.90	23.9	23.4	19.8	20.6
Glucose (mg/dl)	91	85	90	86	97	90	109	107	98	97
Insulin (μU/ml)	12.43	11.42	16.68	18.96	5.66	5.61	15.24	13.03	11.35	10.45
Leptin (ng/dl)	7.74	4.45	8.82	7.37	3.94	3.09	6.12	4.11	1.56	1.75
NE (nmol/L)	0.26	1.00	3.73	2.93	1.00	0.6	1.13	1.47	2.21	2.1

FM: fat mass, NE: Norepinephrine

Table 3. Exercise training data: total training energy expenditure, number of training sessions, changes in power output and VO2 peak.

	S1		S2		S3		S4		S6		Mean±SE	
Total training EE (kcal)	6144		9108		7344		6369		6432		7079±547.0	
Total training sessions	32		36		48		33		32		36±3	
EE/session (kcal)	192		253		153		193		201		199±16	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Power output (w)	37.2	63.1	35.0	46.7	35.7	49.1	33.8	68.5	37.1	52.2	35.8±.6	55.9±4.2*
VO2 peak (l/min)	1.47	1.74	1.85	1.78	1.59	1.41	1.43	1.43	1.45	1.73	1.56±.07	1.62±.08

Data are the mean ± SE. *P<0.05. EE: energy expenditure. Total training sessions: total number of training sessions subjects completed.

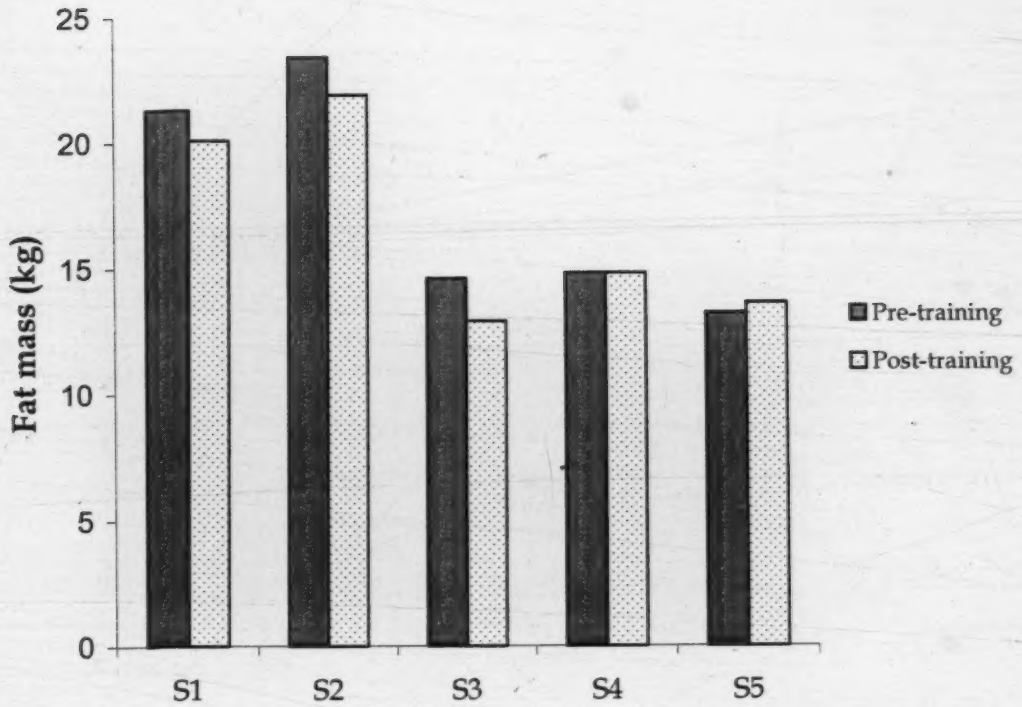


Figure 1. Effects of 12 weeks of FES-Rowing on FM in people with high lesion (above T6) spinal cord injury (N=5).

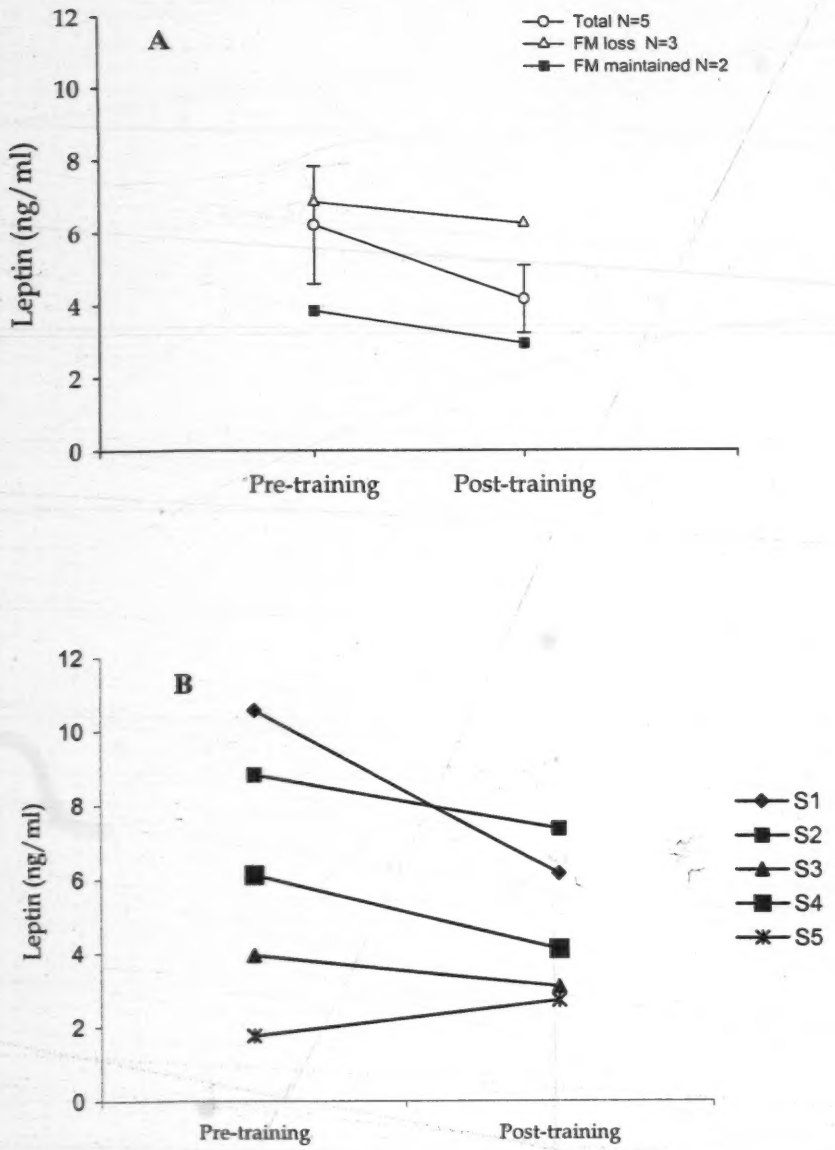
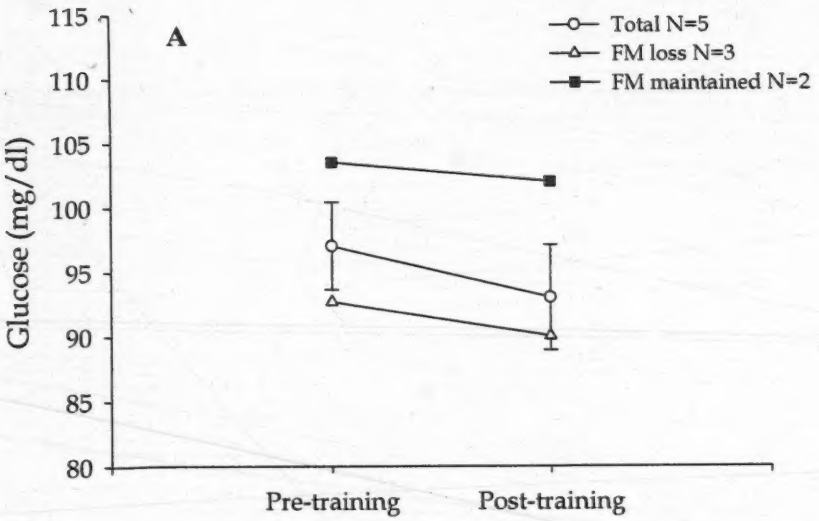


Figure 2. Effects of 12 weeks of FES-Rowing on plasma leptin in people with high lesion (above T6) spinal cord injury (N=5). Subjects trained three to four times per week at 80-90% VO2 peak. A: mean pre- and post-training values for all subjects (N=5), FM loss (N=3), FM maintained (N=2) group. Bars represent 1 standard error on either side of the mean. B: FM values for each subject.



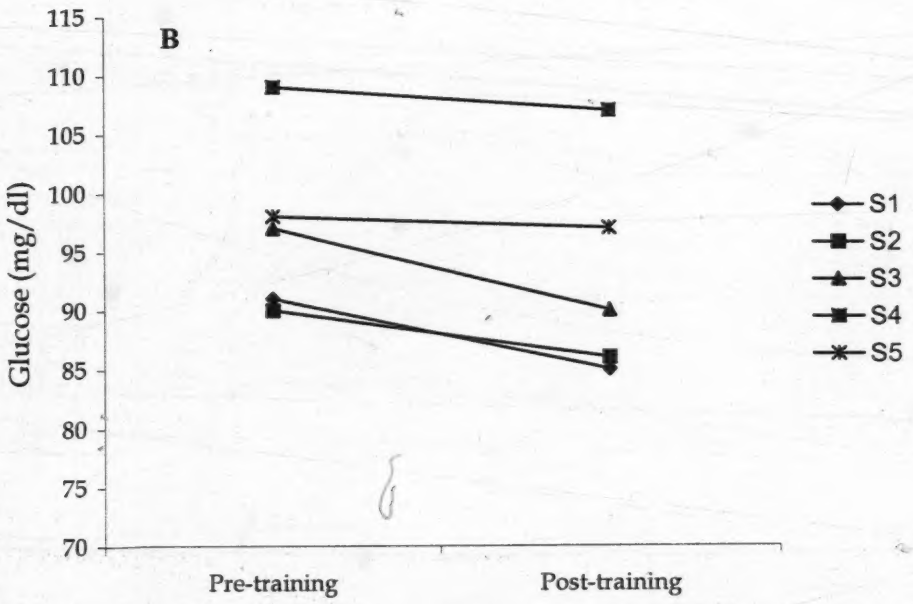


Figure 3. Effects of 12 weeks of FES-Rowing on plasma glucose in people with high lesion (above T6) spinal cord injury (N=5). Subjects trained three to four times per week at 80-90% VO2 peak. A: mean pre- and post-training glucose values for all subjects (N=5), FM loss (N=3), FM maintained (N=2) group. Bars represent 1 standard error ofneither side of the mean. B: plasma glucose levels for each subject.

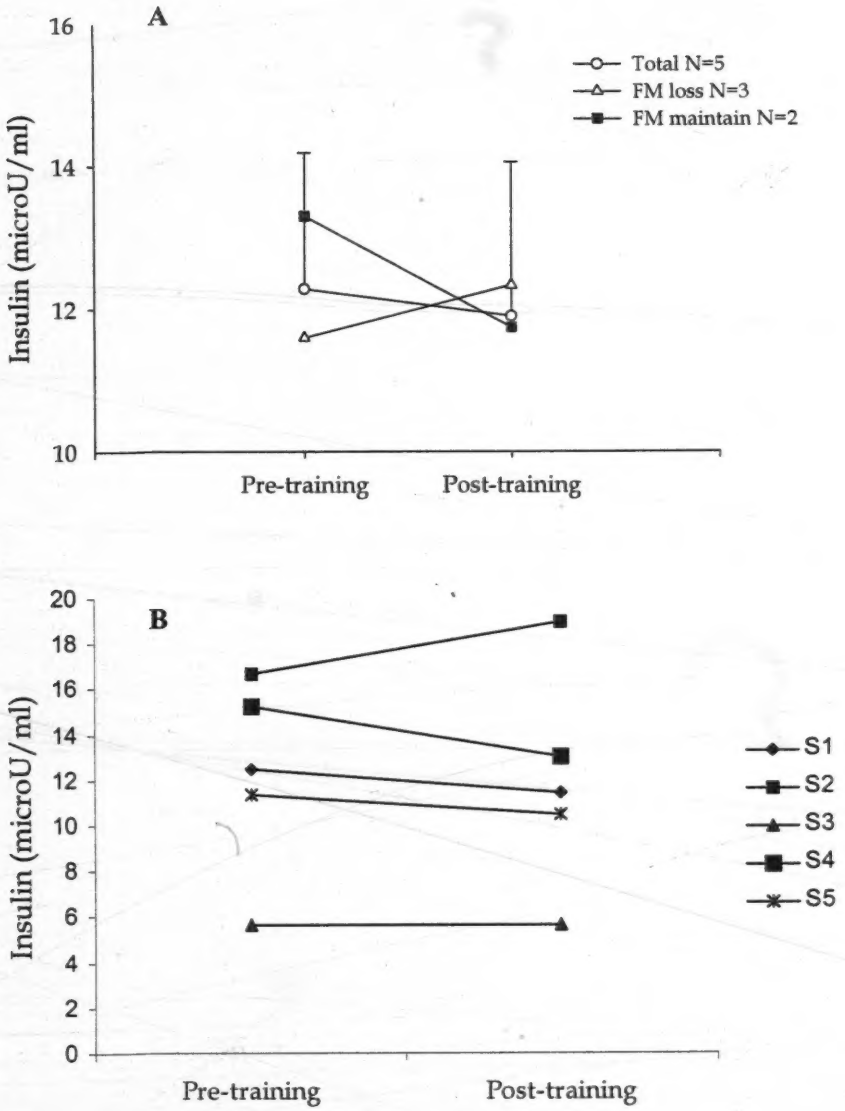


Figure 4. Effects of 12 weeks of FES-Rowing on plasma insulin in people with high lesion (above T6) spinal cord injury (N=5). Subjects trained three to four times per week at 80-90% VO2 peak. A: pre- and post-training values for all subjects (N=5), FM loss (N=3), FM maintained (N=2) group. Bars represent 1 standard error above the mean. B: plasma insulin values for each subject.

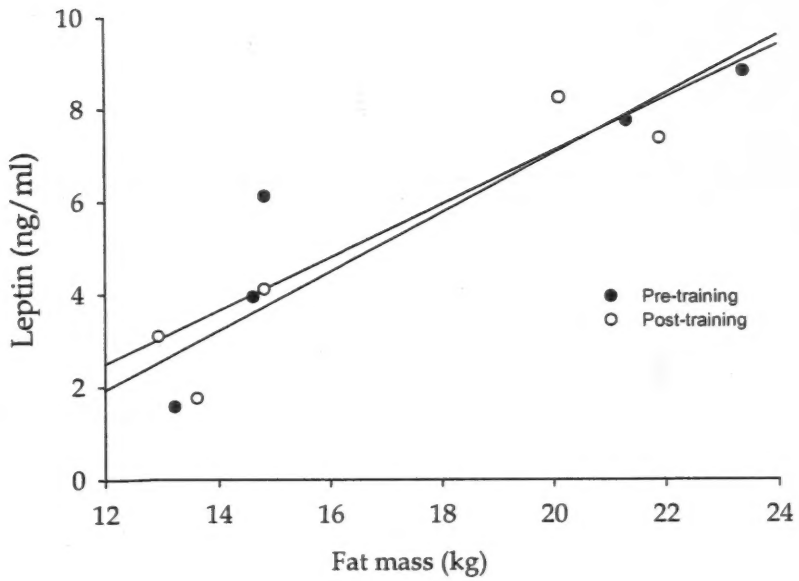


Figure 5. Correlation between fat mass and plasma leptin in people with high lesion SCI pre- and post-training. Plasma leptin levels correlate with FM (Pre-training: r=.90, P=0.04, Post-training: r=.90, P=0.037)

CHAPTER SIX

SUMMARY, CONCLUSION AND FUTURE RECOMMENDATION

SUMMARY

People with a spinal cord injury (SCI) have a three to five times higher risk of developing type 2 Diabetes Mellitus (DM) compared to the general population (2). The higher risk of developing type 2 DM in people with SCI is in part due to increased occurrence of obesity in this population. Elevated fat mass and decreased fat free mass are well documented among people with SCI (8, 13). Obesity, especially extensive visceral adipose deposition, is associated with resistance to insulin-stimulated glucose uptake and may ultimately lead to the development of type 2 DM (3, 4, 9, 15). To this end, leptin, the product of the *ob* gene, has recently received considerable attention because its administration has been shown to reduce fat mass, food intake, hyperglycemia and hyperinsulinemia (1). It is believed that the sympathetic nervous system (SNS) plays a key role in the synthesis and metabolic effects of leptin (5, 12, 14). The SNS is impaired in people with SCI, which may cause altered metabolic and hormonal responses to physiological challenges (10, 11). Consequently, leptin function may be impaired (leptin resistance) and its response to a physiological challenge may be altered in people with SCI. Therefore, loss of SNS function may be related to higher occurrences of obesity in people with SCI. However, plasma leptin levels at rest, in response to fasting and exercise have not been investigated in people with high lesion SCI.

Study one investigated the relationship between circulating leptin levels, body composition and resting metabolic rate (RMR) in people with high lesion SCI (injury level above C7) in body mass index (BMI), weight, and waist circumference (WC) matched able-bodied (AB) subjects. The study showed that people with SCI had 105% higher plasma leptin levels compared to the AB group ($P<0.05$). The presence of a tonic inhibitory adrenergic influence on leptin secretion and the removal of this inhibition, which we observe in people with SCI, would likely contribute to increased plasma leptin levels. Supposedly, elevated plasma leptin levels are associated with increased RMR in the general population but plasma leptin levels correlated with RMR only in the AB group, not in the SCI group. Thus, the loss of SNS-mediated RMR regulation in people with SCI may increase the risk of weight gain. This result may help explain the greater prevalence of obesity in people with SCI. This study was the first study that

investigated the role of SNS in leptin regulation and its function in regulating RMR in human.

Study two was performed to investigate the plasma leptin response to short-term fasting in people with SCI (above C7) compared to AB subjects with normal SNS function. The results showed that plasma leptin levels significantly decreased during 36h fasting by 38.6% and 48.8% in SCI and AB, respectively. The decline in plasma leptin was observed in the AB group at 12 hr but not until 24 hr in the SCI group. The delayed leptin response to fasting in the SCI group may be due to increased fat mass and SNS dysfunction. Despite this delay, it is noteworthy that plasma leptin was still reduced during food restriction in people with SCI. This study demonstrated that people with SCI still have the ability to reduce plasma leptin levels during energy imbalance, which may be regulated by plasma glucose or insulin levels. However, loss of leptin's function in regulating RMR in SCI would make people with SCI less tolerant to an energy deficiency. Leptin's impact on food intake was not investigated in this study.

Knowing that exercise increases energy expenditure and decreases body fat mass (6), exercise is important in people with SCI who already have a higher risk of developing obesity due to impairment of SNS. Study 3 investigated the effects of 12 weeks of FES-Rowing training on plasma leptin, glucose, insulin, norepinephrine and body composition in people with SCI (T6). This study was limited due to a small sample size (N=5), yet we observed a statistically significant decline in fasted plasma glucose levels. Reduction in plasma glucose levels is clinically important since plasma glucose levels are well known markers of glucose metabolism and type 2 DM. Although not significant, plasma leptin (33%, P=0.78) and body fat mass (4.58%, P=0.14) declined. Reduction in fat mass is clinically important since body fat mass is an important risk factor of type 2 DM (7). Although a decline in fat mass would parallel a drop in plasma leptin levels, exercise-induced changes to skeletal muscle leptin sensitivity may also contribute to changes in circulating leptin. Skeletal muscle leptin sensitivity was not assessed in this study and needs further investigation. The number of subjects completing this study was small and statistical significance was not obtained yet (P=0.078), positive changes in plasma glucose, fat mass and plasma leptin levels were

documented after the exercise training. Therefore, exercise is recommended to people with SCI to prevent obesity and its related metabolic disorders, including type 2 DM.

Although subjects from studies 1-3 had injury levels above T6, the subjects from study 1 and 2 had injury levels above C7 while the subjects from study 3 had lower injury levels (T4-5). Subjects from study 3 also had less FM compared to subjects from study 1 and 2. Reduced levels of FM in study 3 was accompanied by reduced resting plasma leptin levels compared to subjects from study 1 and 2. Lower levels of FM and plasma leptin in study 3 could be attributed to the different levels of injury. Those individuals with higher lesion levels may have reduced SNS activity, which may decrease the tonic inhibitory adrenergic influence on leptin production and ultimately increase the risk of developing obesity (9).

CONCLUSION

In conclusion, people with SCI have elevated plasma leptin levels compared to the AB population; and have lost leptin's function to regulate RMR due to impaired SNS. As a result of these changes, the risk of developing obesity may be increased. Combined with our previous findings that exercise with FES-cycling (3 days/week, 30 min/day) improves glucose tolerance and insulin sensitivity (8) study 3 demonstrates the possible benefits of exercise in this population as their plasma leptin, plasma glucose and fat mass were reduced after 12 weeks of FES-Rowing training. Therefore, regular exercise training is recommended for people with SCI to alleviate increased FM, obesity and obesity-related disorders.

In addition, these studies demonstrate that the SNS plays a key role in leptin's influence on RMR and its response to fasting. Therefore, loss of normal SNS function may further increase the risk of developing obesity and decrease tolerance to hypoglycemia.

FUTURE RECOMMENDATIONS

The studies reported in this thesis are the first to describe the leptin response to varying conditions. Other areas that would be of value to investigate in SCI include:

1. Leptin's response to an acute bout of exercise: Differences in exercise-induced energy expenditure or differences in exercise duration and intensity may elicit a different leptin response.
2. Exercise training prescription and dietary modification: Exercise programs need further development to determine the most appropriate and beneficial dose for people with SCI. As well, the combined effects of exercise and dietary intake on FM management need further work to define the impact on insulin sensitivity and risk for developing type 2 DM.
3. Skeletal muscle leptin sensitivity after exercise training: The effects of exercise training on leptin sensitivity have not been established. Improvement in skeletal muscle leptin sensitivity would further decrease TG content and improve insulin sensitivity in skeletal muscle, which decrease the risk of developing type 2 DM.

REFERENCE

1. Ahima RS, Flier JS. 2000 Leptin. Annu Rev Physiol. 62:413-437.
2. Bauman WA, Spungen AM. 1994. Disorder of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: A model of premature aging. Metabolism. 43:749-756.
3. Bjorntorp P, 1990 Portal adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 10:439-496.
4. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, et al. 1997 Body fat distribution and risk of noninsulin-dependent diabetes mellitus in women. The Nurse's Health Study. Am J Epidemiol. 145:614-619.
5. Haque MS, Minokoshi Y, Hamai M, Iwai H, Horiuchi M, Shimazu T. 1999 Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. Diabetes. 48:176-1712.
6. Hjeltnes N, Aksnes AK, Birkeland KI, Johansen J, Lannem A, Wallberg-Henriksson H. 1997 Improved body composition after 8 wk of electrically stimulated leg cycling in tetraplegic patients Am J Physiol 273: R1072-R1079.
7. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Wllett WC. Diet Lifestyle, and the risk of type 2 diabetes mellitus in women. New Eng J Med 345: 790-797, 2001.
8. Jeon JY, Weiss BC, Steadward RD, Ryan E, Burnham R, Bell G, Chilibeck P, Wheeler GD. Improved glucose tolerance and insulin sensitivity after electrical stimulation-assisted cycling in people with spinal cord injury. Spinal Cord 40:110-117, 2002.
9. Jeon YJ, Harber VJ, Steadward RD. (2002) Leptin response to short term fasting in sympathectomized men- role of sympathetic nervous system. American Journal of Physiology: Endocrinology and Metabolism 284:E634-E640.
10. Kjaer M Pollack SF, Mohr T. et al. 1996 Regulation of glucose turnover and hormonal responses during electrical cycling in tetraplegic humans. Am J Physiol 271:R191-R199.
11. Kjaer M, Dela F, Sorensen FB. et al. 2001 Fatty acid kinetics and carbohydrate metabolism during electrical exercise in spinal cord injured human. Am J Physiol. 281:R1492-R1498.

12. Minokoshi Y, Haque MS, Shimazu T. 1999 Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes. 48:287-291.
13. Olle MM, Pivarnik JM, Klish WJ, Morrow JR. 1993. Body composition of sedentary and physically active spinal cord injured individuals estimated from total body electrical conductivity. Arc Phy Med Rehab 74:706-710.
14. Pinkney JH, Coppack SW, Mohamed-Ali V. 1998 Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol 48:407-411.
15. Roden M, Price TB, Perseghin G, et al. 2001 Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest. 97:2859-2865.

APPENDIX A: SUBJECT DATA (DATA EXCLUDED FROM GROUP ANALYSIS)

Rational:

The data from one subject were not included in the group analysis due to the subject's lack of adherence to our training program. The total number of days for this subject to complete the assigned training program was 152 days while the average total number of days for other subjects was 101 days.

Training data

Total training EE (kcal)	975	
Total training session	36	
EE/session (kcal)	183	
	Pre	Post
Power output (w)	30.72	62.82
VO2 peak (l/min)	1.52	1.96

Subject characteristics

Subject	Age (yr)	Height (cm)	Years since injury (yr)	Injury level	ASIA class
6	23	186	1.5	T4	A

Physical, metabolic and hormonal parameters pre- and post-training

	Pre	Post
Weight (kg)	82.1	91.3
Lean mass (kg)	59.4	63.2
FM (Kg)	22.4	25.2
Percent fat (%)	26.5	27.7
Glucose (mg/dl)	91	134
Insulin (μU/ml)	8.46	18.64
Leptin (mg/dl)	6.3	12.84
NE (nmol/L)	1.46	0.79

APPENDIX B: PARTICIPANT INFORMATION LETTER FOR STUDY ONE

PARTICIPANT INFORMATION LETTER

Level of plasma leptin, insulin, cortisol and catecholamine in people with high lesion spinal cord injury

Investigators:
Justin Jeon, MSC Vicki Harber, Ph.D Robert Steadward, Ph.D

What are we doing in this study?

We are examining the levels of plasma leptin, other related hormone levels and body composition in people with high lesion spinal cord injury (SCI) at resting.

Background:

Individuals with SCI undergo abrupt changes in body composition as a result of their injury. These changes include a reduction in muscle mass, bone density, and an increase in fat mass. Altered body composition after SCI put them at a higher risk of developing obesity-related disorders such as cardiovascular disease and diabetes mellitus (DM). Research studies have demonstrated that people with SCI have three to five time the higher risk of developing DM.

Leptin is a newly found hormone produced by fat cell, and its blood levels are strongly correlated with fat mass. Leptin has a role in controlling food intake, energy expenditure and body weight. Absence or lower level of leptin has been shown to cause obesity in animals and humans. This hormone has a physiological function mediated by sympathetic nervous system. Unfortunately, people with high lesion SCI have impaired sympathetic nerve activity due to their injury, which results in the interruption of signals between the brain and sympathetic nervous system. Knowing that the sympathetic nervous system plays a key role in leptin regulation of food intake and energy expenditure, it is important to determine how leptin regulate in people with high lesion SCI in resting and fasting. To our knowledge, there has not been study in which the level of plasma leptin and sympathetic nerve activity have been measured in people with high lesion SCI at rest and fasting. Therefore, the purpose of the study is to determine the level of blood leptin and sympathetic nerve acitivity in people with SCI at resting.

Procedure:

A medical doctor will perform an examination to determine your injury level. To be eligible for the study, the injury level has to be higher than T1.

Day 1.
You will be asked to abstain from any streneous physical activity during the period of study.

24 hours urine samples will be collected for catecholamine determination starting in the morning of Day 1. We will take 10 ml of blood for each sample.

5:00pm – Height and weight will be measured.
6:00pm – You will have a standard meal which will be provided.
8:00pm – Blood collections

Day 2.
8:00am – Blood collection (12 hour fasting) /Returning 24h Urine sample of Day 1.
8:15am – Measuring Resting Metabolic Rate (1 hour)

During over night fasting, you will have a free access to water at any time. Body composition will be measured with in three days prior to Day 1 of the study.

Blood collection:
At 6:00pm of Day 1, you will consume a controlled standard diet. At Day 2, after overnight fast you will be asked to come to the lab and the first blood sample will be collected.

Resting metabolic rate:
Resting metabolic rate will be measured as soon as the blood sampling is completed at 8:00am of Day 2. A transparent canopy will be placed over your head while oxygen consumption is measured over the next 30 minutes. You will not be permitted to sleep or move during the final 30 minute period. This will take 60 min for the measurement.

Body composition measurement:
Body composition will be measured by dual-energy X-ray absorptiometry. You will be required to come to the lab with light clothes. You will be asked to lie down on the testing bed and whole body scan will be performed. This test will take 15 minutes.

Benefits:
Benefits as you participate in the study include:

1. Knowing your percent body fat and bone density.
2. Diagnosis of diabetes. We will be measuring your glucose and insulin levels.
3. General advice on exercise and diet.

4. Your participation will contribute to enhance the knowledge and understanding of diabetes and cardiovascular disease in people with spinal cord injury.

Potential Risk:

Blood collection procedure may cause (very slight chance) infection, bruising and hematoma (bleeding) at the site if not properly cared for. Standard laboratory safety procedures are in place and will be adhere to.

Confidentiality:

To ensure confidentiality, personal information will be coded and stored in a locked file cabinet to which only the investigators whose names are written on this letter will have access. Normally, information is retained for a period of five years post publication, after which it will be destroyed.

Third Party Contact:

If you wish to speak with someone who is not involved with this study, please call Dr. Debra Shogan, Associate Dean (Research and Graduate Studies), Faculty of Physical Education and Recreation, at 492-5910.

Voluntary Participation:

You are free to withdraw from the study at any time without explanation.

CONSENT TEMPLATE
Leptin study

Part 1 (to be completed by the Principal Investigator):

Title of Project: ***Levels of leptin, insulin, cortisol and catecholamine in people with high lesion spinal cord injury***

Principal Investigator: Justin Yong Jeon

Co-Investigators: Dr. Vicki Harber and Dr. Robert Steadward

Part 2 (to be completed by the research subject):

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw from the study at any time without consequence? You do not have to give a reason.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your records?	Yes	No

This study was explained to me by: _____

I agree to take part in this study.

_____ _____ _____
Signature of Research Participant Date Witness

Printed Name_____ Printed Name_____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____
Signature of Investigator or Designee Date

APPENDIX C: PARTICIPANT INFORMATION LETTER OF STUDY TWO

PARTICIPANT INFORMATION LETTER

Effects of fasting on leptin, insulin, cortisol and catecholamine in people with high lesion spinal cord injury

Investigators:
Justin Jeon, MSC Vicki Harber, Ph.D Robert Steadward, Ph.D

What are we doing in this study?

We are examining the levels of plasma leptin, other related hormones and body composition in men with high lesion spinal cord injury (SCI) and able-bodied men at rest and after 36 hours of fasting.

Background:

Individuals with SCI undergo abrupt changes in body composition as a result of their injury. These changes include a reduction in muscle mass, bone density, and an increase in fat mass. Altered body composition following SCI increases the risk of developing obesity-related disorders such as cardiovascular disease and diabetes. Research studies have demonstrated that people with SCI have three to five times the risk of developing diabetes.

Leptin is a newly found hormone produced by fat cells, and its blood levels are strongly correlated with fat mass. Leptin has a role in controlling food intake, energy expenditure and body weight. Lower levels of leptin have been linked with obesity in animals and humans. Leptin requires the sympathetic nervous system to perform its function. Unfortunately, people with high lesion SCI have impaired sympathetic nervous system due to their injury, which results in the interruption of signals between the brain and sympathetic nervous system. Knowing that the sympathetic nervous system plays a key role in leptin regulation of food intake and energy expenditure, it is important to determine how leptin is regulated in people with high lesion SCI at rest and fasting. To our knowledge, there has not been a study in which the level of leptin and sympathetic nervous system has been measured in people with high lesion SCI. Therefore, the purpose of this study is to determine the level of leptin and sympathetic nervous system function in people with SCI at rest and after a 36 hour fast.

Procedure:

To be eligible for the study, the injury level has to be higher than C7 for the participants with SCI.

You will abstain from alcohol consumption and strenuous physical activity during the three day period of study.

Two 24 hour urine collection, four 10ml (less than the amount of table spoon) blood samples and two resting metabolic rate measurements will be obtained from you over a three day period.

Total time required in participating in the study is approximately 6.5 hours. **It is required for the participants to fast for 36 hours during the study**

Day 1.

Following the first void – 1st 24-hour urine collection begin.

6:00pm – You will have a standard meal (800 kcal, 50% carbohydrate, 35% fat, 15% protein) which will be provided.

9:00pm – Blood collections (10 ml)

You will not be allowed to eat any food except water after 9:00pm.

Day 2. (Fasting)
You will be asked to fast all day of Day 2. You will have free access to water.

Completion of 24-h urine collection for Day 1.

Following the first void- 2nd 24-h urine collection begin.

8:00am – Measuring resting metabolic rate (1 hour)

9:00am – Blood collection (10 ml/12 hour fasting)

9:00pm – Blood collection (10ml/24 hour fasting)

Day 3.
Completion of 24-h urine collection for Day 2.
8:00am – Resting metabolic rate will be measured (1hour)

9:00am – Blood collection (36 hour fasting)

9:15am – Study complete

You are not allow to bike or run to come to the lab when you are to measure your resting metabolic rate in the mornings of Day 2 and 3 of the study.

Percent body fat and bone density will be measured within three days of Day 1 of the study through dual-energy x-ray absorptiometry (DEXA). You will have free access to water during the study.

Blood collection (4 blood samples will be obtained):
At 6:00pm of Day 1, you will consume a controlled standard diet. At 9:00pm of Day 1, the first blood sample will be taken. At day 2, after an overnight fast, you will be asked to come to the lab (8:00am) and the second blood sample will be collected after resting metabolic measurement. At 9:00pm of day 2, the third blood sample will be taken. At 9:00am of day 3, the last blood sample will be taken from you.

Resting metabolic rate (twice):
Resting metabolic rate will be measured at 8:00am of Day 2 and 8:00am of Day 3. You will rest in a lying position for 30 minutes prior to measurement. A transparent canopy will be placed over your head while oxygen consumption is measured over the next 30 minutes. You will not be permitted to sleep or move during the final 30 minute period. This will take 60 min for each measurement.

Body composition measurement:
Body composition will be measured by DEXA. You will be required to come to the College Plaza Medical Imaging Center. You will be asked to lie down on the padded testing bed and whole body scan will be performed. This test will take less than 15 minutes.

Benefits:
Benefits for participants in this study include:

5. Knowing your percent body fat and bone density.
6. General advice on exercise and diet.
7. Your participation will contribute to enhance the knowledge and understanding of diabetes and cardiovascular disease in people with spinal cord injury.

Potential Risk:
The blood collection procedure may cause (very slight chance) infection, bruising and bleeding at the site if not properly cared for. Standard laboratory safety procedures are in place and will be adhered to. You may experience some discomfort and hunger due to the 36-h fast.

Confidentiality:
To ensure anonymity, personal information will be coded and stored in a locked file cabinet to which only the investigators whose names are written on this letter will have access. Normally, information is retained for a period of five years post publication, after which it will be destroyed.

Third Party Contact:

If you wish to speak with someone who is not involved with this study, please call Dr. Debra Shogan, Associate Dean (Research and Graduate Studies), Faculty of Physical Education and Recreation, at 492-5910.

Voluntary Participation:

You are free to withdraw from the study at any time without explanation or consequences.

CONSENT TEMPLATE
Leptin study

Part 1 (to be completed by the Principal Investigator):

Title of Project: ***Effects of fasting on leptin, insulin, cortisol and catecholamine in people with high lesion spinal cord injury***

Principal Investigator: Justin Yong Jeon

Co-Investigators: Dr. Vicki Harber and Dr. Robert Steadward

Part 2 (to be completed by the research subject):

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw from the study at any time without consequence? You do not have to give a reason.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your records?	Yes	No

This study was explained to me by: _____

I agree to take part in this study.

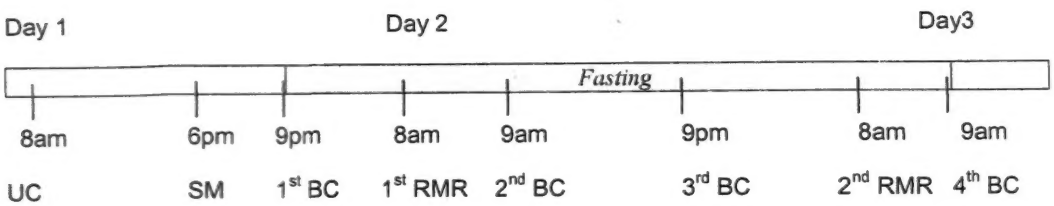
_____ _____ _____
Signature of Research Participant Date Witness

Printed Name_____ Printed Name_____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____
Signature of Investigator or Designee Date

Figure 1. Timeline for procedures



Acronyms:

SM: Standard Meal
BC: Blood Collection
RMR: Resting Metabolic Rate Measurement

APPENDIX D: PARTICIPATION INFORMATION LETTER FOR STUDY THREE

PARTICIPANT INFORMATION LETTER

Effects of 12 weeks of functional electrical stimulation-assisted rowing training on blood lipids in people with spinal cord injury

Investigators:

Robert Steadward, Ph.D Dr. Garry Wheeler

Mr. Research Assistant:
Dries Hettinga
University of Leuven, Belgium
Visiting Masters Degree Student

Purpose

The purpose of this study is to examine the effects of functional electrical stimulation (FES)-assisted rowing training on risk factors for Coronary Heart Disease (CHD) in people with spinal cord injury (SCI) who are in higher risk of developing CHD.

Background:

Individuals with SCI experience changes in body composition as a result of their injury. These changes include a decrease in amount of muscle, bone density (the amount of calcium in the bones), and an increase in amount of body fat. Changes in body composition following SCI increase the risk of developing obesity-related disorders such as CHD and diabetes. Research studies have demonstrated that people with SCI have much higher risk of developing CHD. However, it has been proven that regular exercise is one of the best tools to reduce risk factors for CHD such as cholesterol levels.

Although people with SCI have much higher risk of developing CHD, limited research has been conducted on the effects of exercise on CHD. Part of the reason for lack of exercise-related research in SCI, is the difficulty in exercising hard enough to produce changes in risk factors for heart disease. This is because the large leg muscles are unable to exercise and arm exercise alone is not sufficient to stimulate reductions in risk factors for heart disease. To this end so-called ***hybrid*** exercise systems which combine lower limb exercise with functional electrical stimulation (FES) and upper limb ergometry has been suggested as a possible means of increasing the amount and intensity of exercise in persons with SCI and therefore to hopefully

cause changes in risk factors. Functional electrical stimulation is simply the use of an electrical current applied to paralysed muscle through electrodes placed on the legs for example, to produce muscle contractions. FES-rowing has been developed by Dr. Garry Wheeler, Dr. Brian Andrews and Robert Lederer et al. (2001) at the University of Alberta as one type of hybrid exercise. We plan to examine the effects of FES-Rowing on risk factors for CHD in people with SCI, particularly cholesterol.

Procedure:

To be eligible for the study, your injury level has to be between T12 and T4 and you need to be over 18 years old. We will conduct a full medical examination to ensure no undue risk is associated with your taking part in exercise. You may be asked to undergo additional diagnostics prior to participation, which you may decline and decide not to participate in the study. The training will have two parts.

Phase I:

Phase I is designed to increase basic leg strength and will last two to four weeks depending on the strength of your legs (10 to 20 hours). This will involve the application of electrodes to the thigh area and an electrical current to produce leg extensions against the force of gravity, for a period of up to 10 minutes of continuous exercise. Please note that if you are unable to complete 10 minutes of continuous leg exercise after a total of 4 weeks you will be asked to withdraw from the study. This could happen because the type of spinal injury sometimes results a lack of muscle response to electrical currents.

Phase II:

Once you have completed phase I of the study and can do 10 minutes of continuous leg extension (and flexion) exercise, you will proceed to phase II, which requires you to exercise up to 1.5 hours per day, four times per week for 12 weeks (Total of 72 hours). The total time required in participating study is approximately up to 100 hours over a three month period.

Percent body fat and bone density will be measured before and after the 12 weeks of training by dual-energy x-ray absorptiometry (DEXA).

Blood collection (4 blood samples will be obtained):

Four venous blood samples (14 ml each) will be collected from you before, during and after the 12 weeks of training. One sample will be taken before the study starts; one sample at six weeks, and 2 samples after 20, and 44 hours after the last exercise session at 12 weeks. All the blood samples will be taken after an overnight fast (no food 12hours prior to the exercise session).

Body composition measurement:

Body composition will be measured by a procedure called Dual Energy X-ray absorptiometry (or DEXA for short). To have this X-ray procedure you will be required to come to the College Plaza Medical Imaging Center. You will be asked to lie down on the padded testing bed and whole

body X-ray scan will be performed. This can tell us the density of your bones and also the amount of body fat that you have. This test will take less than 15 minutes. Please note that the total amount of radiation exposure in this procedure is minimal (see below).

Benefits:

Benefits of participants in this study include:

8. Knowing your percent body fat, fitness level and cholesterol level.
9. Familiarity with a new type of exercise opportunity for persons with spinal cord injury.
10. An improvement on your fitness
11. General advice on exercise and diet.
12. Your participation and discussions with the investigators during the study will contribute to, and enhance your knowledge and understanding of the risk factors for CHD and the benefits of exercise for people with spinal cord injury.
13. the health-related benefits which may accumulate during 12 weeks of exercise.

Potential Risk:

The blood collection procedure may cause (very slight chance) infection, bruising and a hematoma (bleeding) at the site if not properly cared for. The research team will take every precaution to ensure that blood is collected in a sterile manner. The skin will be cleaned with alcohol; a new and sealed needle will be used for every sample, and a sterile gauze dressing will be supplied following withdrawal of the sample. It is recommended to apply pressure at the site of blood collection for 5 minutes.

Maximum oxygen uptake will be measured during a progressive, incremental exercise test to a failure point – that is – when you are no longer able to maintain full leg extensions during rowing for three consecutive strokes (leg extension followed by the pull phase of rowing). This test requires maximal effort in order to achieve a peak oxygen consumption value. With this type of exercise there may be some health risk. During and after the tests it is possible to experience symptoms such as abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and in very rare cases (0.5 per 10,000 in testing facilities), heart rhythm disturbances or heart attack. While serious risk to healthy participants is highly unlikely, they must be acknowledged and participants willingly assume the risks associated with very hard exercise. The exercise test will be administered by qualified personnel who are under the supervision of Dr. Garry Wheeler. Personnel understand emergency procedures and are certifidedin CPR.

Risk due to exposure to the DEXA system is minimal and is equivalent to one tenth the X ray energy received during a standard dental X ray examination.

Confidentiality:

To ensure confidentiality, personal information will be coded and stored in a locked file cabinet to which only the investigators whose names are written on this letter will have access. Normally, information is retained for a period of five years post publication, after which it will be destroyed.

Third Party Contact:
If you wish to speak with someone who is not involved with this study, please call Dr. Wendy Rodgers, Chair, Faculty Ethics Committee at 492-5910.

Voluntary Participation:

You are free to withdraw from the study at any time without explanation or penalty. If you want to withdraw from study, simply notify Dr. Garry Wheeler. Contact information for Dr. Wheeler is found at the top of this letter.

CONSENT

Part 1

Title of Project: ***Effects of 12 weeks of FES-Rowing training on blood lipids in people with spinal cord injury***

Principal Investigator: Dr. Garry Wheeler

Co-Investigators: Justin Y. Jeon and Dr. Robert Steadward

Part 2 (to be completed by the research subject):

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw from the study at any time? You do not have to give a reason and it will not affect your care.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your records?	Yes	No

This study was explained to me by: _____

I agree to take part in this study.

_____ _____ _____

Signature of Research Participant Date Witness

Printed Name_____ Printed Name_____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____

Signature of Investigator or Designee Date

All participants in research projects are advised that the information they provide, and any other information gathered for research projects, will be protected and used in compliance with Alberta's Freedom of information and Protection of Privacy Act (FOIPP)